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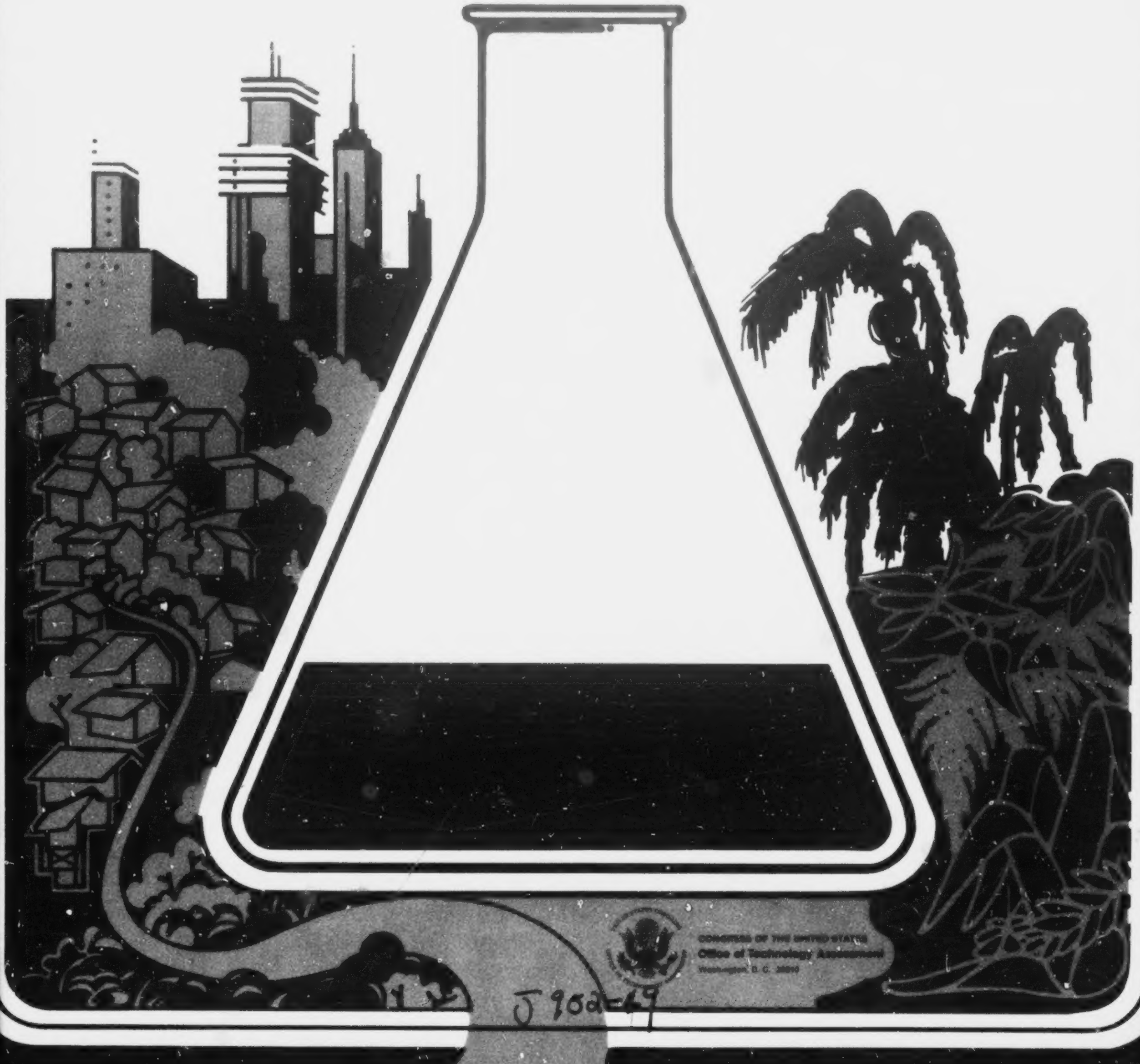

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Status of Biomedical Research and Related Technology for Tropical Diseases

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Foreword

Billions of people in less developed areas of the world and a small but growing number of inhabitants of the industrialized countries come into contact with infectious diseases generally characterized as "tropical." Though not necessarily geographically limited, these diseases are most common in the tropics because of the social and economic, as well as climatic, conditions that prevail there.

In combination with the traditional methods of parasitology and basic research, the new tools of biotechnology and immunology have opened doors to finding methods for controlling tropical diseases. Scientists using both traditional and recombinant DNA techniques are pursuing technologies for the control of insects that transmit diseases and the development of vaccines, diagnostic technologies, and new therapies for tropical diseases. The possibilities for making inroads into the overwhelming morbidity and mortality caused by these diseases have never been so promising.

The United States continues to make significant contributions toward controlling tropical diseases, even though the resources allotted to these efforts are small: in recent years, less than $100 million per year has been spent by the U.S. Government on tropical disease research out of a total annual biomedical research budget of $4 to $5 billion. As one component of U.S. international activities, research in tropical diseases serves several objectives: social and humanitarian, political, economic, medical (including the protection of American citizens), and scientific. Opportunities for expanding U.S. influence and contributions in this field are great.

In the spring of 1983, the Senate Appropriations Committee asked OTA to examine the status of biomedical research and technologies for controlling tropical diseases. The request was precipitated by a question about continued funding of the Gorgas Memorial Institute of Tropical and Preventive Medicine, Inc. and its operating arm, the Gorgas Memorial Laboratory in the Republic of Panama. An OTA technical memorandum, *Quality and Relevance of Research and Related Activities at the Gorgas Memorial Laboratory*, was released in August 1983 as a response to the immediate question. The larger question is addressed in this full assessment.

An advisory panel, chaired by Dieter Koch-Weser, provided guidance and assistance during the assessment. A large number of individuals from academia, the Federal Government, the private sector, and the public provided information and reviewed a draft of the report. Five contractors were essential to the completion of the assessment: Paul F. Basch surveyed the use of biotechnology in tropical disease research; Veronica Elliott compiled information about funding; Roger A. Bitar contributed the material on therapies for tropical diseases; and Myron M. Levine and Lydia Schindler prepared case studies on oral rehydration therapy for diarrheal diseases and on the development of a malaria vaccine, respectively.

The final responsibility for the content of the report rests with OTA. Key staff involved in the analysis and writing were Hellen Gelband, Kerry B. Kemp, Susan C. Tripp, and Steven S. Bjorge.

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Findings and Options

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1.
Findings and Options

INTRODUCTION

For most of the world's population in less developed countries, mainly in the tropics, good health is not something interrupted by occasional annoying bouts of sickness. One-tenth of the average person's life in a developing country is seriously disrupted by ill health (412). Life expectancy at birth is nearly 20 years shorter in the developing regions than it is in the developed countries. Each year, millions of infants and children die from enteric and respiratory infections. Several hundred million people are infected with organisms that cause chronic disabling and often life-threatening parasitic diseases: malaria, schistosomiasis, trypanosomiasis, leishmaniasis, and filariasis. **Chronic and debilitating infections with intestinal parasites range, in total, into the billions.**

Researchers in all corners of the globe have aided the efforts against tropical diseases, and the United States has played a major role. Through the work of individual U.S. researchers and institutions and through international collaborations, the United States has contributed to the development of oral rehydration therapy (ORT) for the dehydration that accompanies diarrheal diseases, the rapidly unfolding progress toward de-

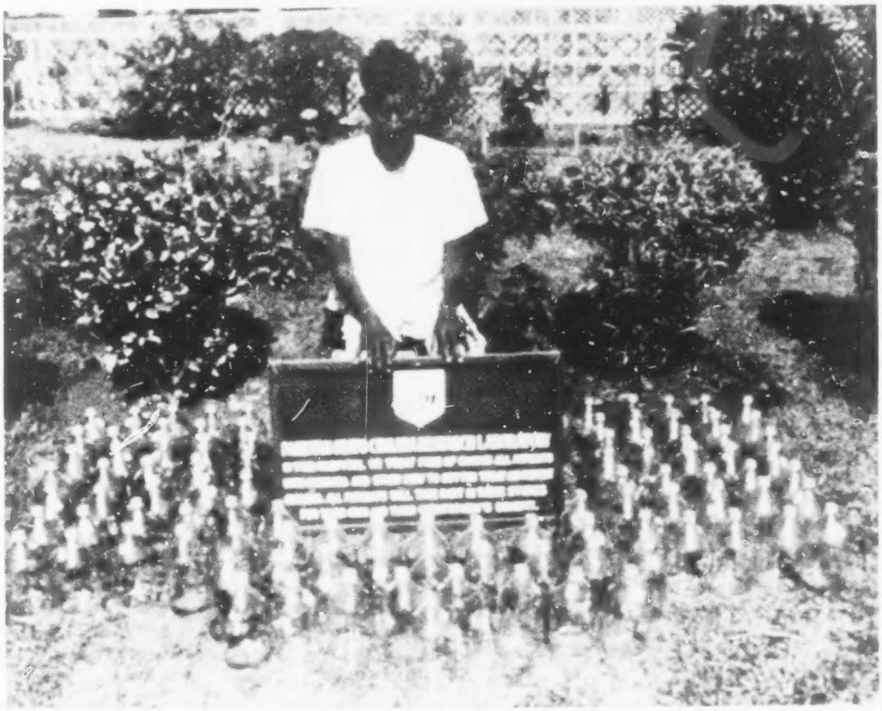

Photo credit: Dr. Robert Edelman, National Institutes of Health

A cholera patient surrounded by bottles that contained the 96 liters of intravenous fluid required for his recovery. Oral rehydration therapy (ORT) has cut the requirement for expensive intravenous fluid by about 75 percent.

velopment of a genetically engineered malaria vaccine, and other advances in the prevention, diagnosis, and treatment of tropical diseases.

ORT and the malaria vaccine fall at opposite poles of biomedical research and of disease interventions. ORT, developed from research in basic human physiology, has saved the lives of hundreds of thousands of children by treating the lethal dehydration of diarrhea caused by a plethora of organisms. The hope for a malaria vaccine is to prevent at least some part of the 300 million cases that occur worldwide each year. Widespread application of an effective vaccine, still at best 5 to 10 years away, will be dependent on the use of recombinant DNA technology, a tool that did not even exist 20 years ago. These advances are undeniably valuable contributions, both for science and for health. But even these achievements pale in the shadow of the massive health problems that continue to affect the billion or so who live in less developed areas.

This assessment examines the status of control for a selected group of diseases that cause extensive morbidity and mortality in developing countries of the tropics and looks at what the United States is doing in biomedical research to alleviate the human misery caused by these diseases. **OTA found a great deal of recent progress in tropical disease research and a small, but highly motivated, corps of tropical disease researchers.** OTA also found, however, that the U.S. Government is spending relatively little money on tropical disease research. **The small amount of money spent by the United States on tropical disease research appears to reflect choices in policy, whether implicit or explicit, and not a lack of promising avenues for research.**

Request for the Assessment

The need for an assessment of research in tropical diseases was focused in the spring of 1983, when a question arose about continued U.S. funding for Gorgas Memorial Laboratory (GML), a half-century old research institution located in the Republic of Panama. The National Institutes of Health (NIH) requested no funds for GML for fiscal year 1984. If acted upon, the withdrawal of funding would probably have forced the laboratory's closing.

The Senate Appropriations Committee asked OTA to assess the activities of GML and then go on to examine the overall status of U.S.-funded biomedical research for tropical diseases. An OTA technical memorandum fulfilling the first charge, *Quality and Relevance of Research and Related Activities at the Gorgas Memorial Laboratory*, was released in August 1983 (360). On the basis of that report, a companion report prepared by the General Accounting Office (355), and other information, Congress restored funding for GML for fiscal year 1984. While recognizing the creditable record of research and service of GML, Congress also required that plans for the future course of the laboratory's efforts take account of some problems identified by OTA and the General Accounting Office.

Scope of the Report

There are many definitions of tropical diseases. In the strictest sense, tropical diseases are those that occur only in tropical areas, between the Tropics of Cancer and Capricorn, and are limited to those areas by geographic and climatic factors. In the broadest sense, they can include any health conditions that occur in the tropics, regardless of their distribution around the world. A more useful definition includes those diseases or conditions that occur or could occur in many regions, but which are considerably more prevalent in tropical areas because of the social, economic, and climatic conditions that characterize many tropical countries.

In this report, no attempt was made to redefine tropical diseases. **For the purpose of this assessment, OTA focused on a limited group of conditions that are unquestionably important causes of morbidity and mortality and that either occur exclusively in the tropics or have greater public health implications in the tropics than in temperate zones.** Included in this group are the six diseases singled out by the Special Program for Research and Training in Tropical Diseases (TDR) of the World Health Organization (WHO)/U.N. Development Program/World Bank: malaria, schistosomiasis, trypanosomiasis, filariasis, leishmaniasis, and leprosy. Also considered in this report are tuberculosis, diarrheal diseases, acute respiratory infections (ARIs), and arboviral and related viral infections.

The focus of this assessment is on biomedical laboratory research pertaining to these diseases and on some field research in the control of insect and other vectors. In general, this report does not address the equally important contributions of entomological research and basic ecological research necessary to improve the understanding of these diseases. It also does not examine research in human behavior and societies or the interactions of humans with the environment, elements that are of great importance in understanding and controlling disease.

Research, which provides the knowledge and tools that can be applied to control tropical diseases, is only one arm of the triad that makes up the U.S. effort in international health. Two other arms of the triad—application and training—are not the focus of this assessment but are also deserving of attention.

Application of the knowledge and tools developed through research is aimed at directly improving health conditions. Millions of deaths still occur as a result of diseases for which control measures are available, but are not applied.

Training yields the personnel both for research and for the application of biomedical technologies in the field. There is widespread concern among medical and scientific personnel in tropical health that a crisis exists in the dwindling U.S. capacity for training in this area. The National Research Council (NRC) of the National Academy of Sciences (NAS) has recently begun an assessment of the capacity of U.S. institutions to train personnel and carry out research in tropical medicine. It will also examine different types of funding mechanisms and programs for tropical health research. NRC's report is scheduled for completion in 1985.

Structure of the Report

This report examines the U.S. role in and contributions to biomedical research in selected tropical diseases, and assesses the status of biomedical technologies for controlling those diseases. The remainder of this chapter presents the major findings of the assessment along with some background material, identifies issues, and develops options to address those issues.

Chapter 2 discusses the rationale for U.S. involvement in international health activities and reviews some important reports and legislation that have shaped the U.S. role.

Chapter 3 analyzes funding for tropical disease research, focusing on funding from the U.S. Government, from TDR, and from WHO. It also considers the contributions of foundations and pharmaceutical companies.

Chapters 4 through 9 summarize the status of biomedical technologies for tropical diseases and review current advances from research. Chapter 4 briefly describes the diseases or classes of diseases covered—malaria, schistosomiasis, trypanosomiasis, leishmaniasis, filariasis, leprosy, tuberculosis, diarrheal diseases, ARIs, and arboviral and related diseases—and reports advances in basic knowledge about the disease organisms.

Chapter 5 describes the overall strategies for tropical disease control programs. Chapter 6 treats vector control technologies; chapter 7, immunization technologies; chapter 8, diagnostic technologies; and chapter 9, therapeutic technologies.

Case studies of the development of two technologies are presented following chapter 9. Case study A is about ORT for diarrheal disease, and case study B is about the development, up to the present, of vaccines to prevent malaria.

Summary of Options Developed in This Assessment

The options that follow are discussed in the text.

Medical Technology Development (pp. 19-21)

- Explicitly include drugs and vaccines for tropical diseases in the definition of "orphan drugs" under the Orphan Drug Act of 1983 (Public Law 97-414).
- Encourage Federal agencies, such as the U.S. Agency for International Development (AID),

to examine the possibility of interesting private companies in developing medical technologies for tropical diseases by guaranteeing purchases of products and assisting in field trials.

- Mandate the creation of and authorize funds for a quasi-governmental nonprofit corporation to undertake research and development of medical technologies for tropical diseases until the technologies become economically attractive enough for private industry to take over with the right to an exclusive license for the product.

OR

Stimulate the development of an international nonprofit corporation, funded through contributions from the U.S. Government, other governments, and international bodies, to undertake such research and development.

OR

Create a nonprofit corporation charged with ensuring the development and availability for use in developing countries of prophylactic and therapeutic agents for which there appears to be insufficient commercial interest.

Information for Congressional Decisions (pp. 21-23)

- Hold a special appropriations hearing for tropical disease research with representatives from NIH, the Centers for Disease Control (CDC), the Department of Defense (DOD), and AID, and perhaps invite international agencies and private foundations to participate.

AND/OR

- Require each agency mentioned above to submit a report on the status of its tropical disease research, providing data specified by the Appropriations Committees for use during appropriations hearings.

Research Funding (p. 23)

- Increase Federal funding for all aspects of tropical disease research.
- Amend the international health mandate of the Department of Health and Human Services (DHHS) to remove the limitations on the research DHHS may support in tropical diseases.

BACKGROUND ON TROPICAL DISEASES

Health in the Tropics

In many developing countries of Africa, Asia, Latin America, and other parts of the world, infectious diseases, which have been eliminated as major causes of death in the United States and other developed countries, are the biggest killers. The victims are often infants and children. In the United States, only about 11 of every 1,000 live born babies die in the first year of life, and many of the deaths are attributable to problems evident at birth—low birthweight, premature birth, birth defects, and respiratory problems, for example. In the developing world, as many as 200 of every 1,000 babies born die before their first birthday. The causes are almost entirely infectious diseases—especially diarrheal diseases, ARIs, and malaria. In Latin America and the Caribbean, diarrheal diseases are the leading cause of death in children under 5 years of age (266). **Worldwide, as many *children* die from diarrheal disease, as there are *total* deaths from cancer.** In many places where diarrheal diseases are under better control, ARIs are the main cause of death in children. Although ARIs are common among children in the United States, they rarely are fatal, causing a total of about 5,000 deaths per year (not including influenza).

People in the less developed countries of the tropics are not only afflicted with diseases that seldom occur in temperate regions—e.g., malaria, schistosomiasis, trypanosomiasis, leishmaniasis, and filariasis; they are also afflicted with the diseases of the developed countries in temperate regions. In fact, some types of cancers are more common in less developed countries than they are in the United States or Europe. Certain areas of Central America have the highest known rates of cervical cancer in the world. Parts of Africa and Asia have extremely high rates of liver cancer. Heart disease has become more common in less developed countries as well, particularly in urban areas. Sexually transmitted diseases are more prevalent in many developing countries than they are in the United States.

The presence or absence of good health is determined by forces that interact with the effects of particular agents of disease. Poor nutrition, high birth rates, and lack of education, especially for women, are conditions that exist in the less developed countries with the poorest health. Poverty itself, if defined in terms of per capita income, does not necessarily condemn a country to ill health. In Cuba, for instance, where the average salary is about $200 per month, there has been great emphasis since the 1959 revolution on providing universal access to health care, providing for adequate nutrition, and providing for universal education. Life expectancy in Cuba is now close to that in the United States. The infant mortality rate has been cut from over 100 per 1,000 before the revolution to about 17 per 1,000. Malaria and diphtheria have been eliminated from Cuba. Deaths from diarrheal diseases and tuberculosis, formerly important public health problems, are negligible (316,348).

In many less developed countries, however, malnutrition is a primary factor in disease. Poorly nourished people are more susceptible to disease and often suffer more severe effects when they contract disease. Where the case fatality rate from measles is high, the deaths are generally of undernourished children. The body's immune system and recuperative powers both are diminished by the lack of adequate nutrition. Undernourished pregnant women are of special concern. The babies born to such women are often of low birthweight, which is the main predictor of infant mortality. Those that survive past infancy may themselves be weak and prone to infection. The mothers may be unable to produce sufficient milk to nourish the infants, perpetuating the cycle of undernourishment.

Many scientists believe that the high infant mortality rates associated with undernutrition lead to high birth rates (see case study A). Families who depend on children as workers and for support in old age attempt to have enough babies to ensure the survival of a few. As women undergo pregnancy after pregnancy, they become progressively weaker themselves and have fewer resources for the surviving children. The last children born to them may be the weakest, and most prone to die in infancy or childhood.

Educating women is an important step toward better health. Educated women are better able to learn about the role of nutrition in their own and their children's health and may improve the probability of their children's survival. Though cause and effect would be hard to prove, for each year of schooling for women, the World Bank has estimated that the infant mortality rate is reduced by nearly 1 percent (243). Education also leads to higher expectations for the women and for their children, which promote better health.

The cycle of malnutrition, disease, and high birth rates can be broken in a number of ways. This report focuses on technologies to address the problems of disease directly. A 1982 OTA report (359) considered current and developing technologies for world population and fertility planning. A more recent OTA study addressed technological alternatives to food aid in Africa (363).

Tropical Diseases in the United States

Although some "tropical diseases" are in large part restricted to the tropics because the conditions necessary for their existence have been limited by geography or climate, many "tropical diseases" are not limited by natural factors to the tropics, but also occur in temperate areas, including the temperate United States. The semitropical and tropical areas of the United States are, of course, vulnerable as well.

Diarrheal diseases and ARIs occur in all parts of the world. In the United States, virtually all children survive bouts of diarrhea and colds with little or no long-term consequences. What makes diarrheal illnesses and ARIs important as "tropical diseases" is the fact that infants and children in developing countries commonly die from them.

Tuberculosis was among the leading causes of death in the United States early in this century. Improved living conditions for most Americans decreased transmission of the disease to fairly low levels. Tuberculosis still is widespread in the developing world and wherever substandard, overcrowded living conditions exist. In 1982, more than 25,000 U.S. cases, undoubtedly an underreporting, were registered by CDC. Tuberculosis is common in the home countries of many recent refugees coming to the United States. In 1982, at least 300 refugees entered the United States with active tuberculosis (372).

Malaria was endemic to the United States until its elimination in about 1950, but the conditions for its reestablishment in some parts of the country still exist. In 1982, a total of 1,056 cases of malaria in the United States were reported to CDC (372), fewer than the previous 2 years. All but 17 cases were acquired outside the United States. The 17 cases acquired in the United States were either congenital, associated with blood transfusions, or accidentally acquired in the laboratory. Very recent evidence suggests that malaria caused by *Plasmodium vivax* is being transmitted among farmworkers by mosquitoes in the central valley of California.

Chagas' disease (American trypanosomiasis) has been limited to the New World tropics and never was endemic to the United States. However, the insect vectors of Chagas' disease (reduviid bugs) are present across the Southern United States, and at least some are infected with trypanosomes. A case of Chagas' disease transmitted by an insect in northern California was diagnosed in 1982, the first such case reported in the United States since 1955, when two infants in Texas were diagnosed with domestically acquired Chagas' disease (309).

Four cases of leishmaniasis transmitted in the United States were reported at the 1983 annual meeting of the American Public Health Association (408).

Some of the traditionally tropical arboviruses are extending their ranges within the tropics and to temperate areas, probably aided by the increase in air travel over the past decade. The principal vector of dengue fever and yellow fever, the mosquito *Aedes aegypti*, is common throughout the world, including the Southern United States. In 1982, there were 45 cases of dengue fever confirmed by CDC, in 14 States. Since 1977, 855 suspected cases have been reported, some from almost all the States. Epidemiologic investigations have indicated that most of the infections were contracted outside the United States. More significant though, are the approximately 40 cases of dengue fever contracted in the United States in 1980, the first such reports since 1945. Venezuelan equine encephalitis has been recently introduced into the United States from South America. In other parts of the world, arboviruses, including those that cause Rift Valley fever and African swine fever, also have demonstrated their ability to spread.

Leprosy is transmitted in this country. Since 1970, somewhat fewer than 30 cases have been acquired domestically each year. The total number of cases reported annually in the United States has been increasing, however, because of an increase in cases acquired outside the country. A sharp increase in the number of leprosy cases since the mid-1970s corresponds to the pattern of refugees entering the United States from Southeast Asia (372).

There are a number of ways for "tropical diseases" to become established or reestablished in the United States. Such diseases could be spread into the United States gradually from contiguous geographic areas (e.g., from Latin America through Mexico). They also could be introduced from endemic areas by Americans who have contracted diseases abroad or by foreign visitors or immigrants entering the country. Between fiscal year 1975 and 1982, more than 800,000 refugees, most from Asian areas where tropical diseases are prevalent, entered the United States and have since settled in all parts of the country. A large number of other immigrants also come from tropical areas.

The United States has a relatively strong system of disease surveillance that allows the detection of unusual disease activity in the country and near U.S. borders. However, **the scientific basis for predicting whether a disease is likely to become established or reestablished is fairly weak.**

Climatic conditions do not favor the establishment of some diseases in the United States, and for other diseases (e.g., African sleeping sickness), suitable vectors (tsetse flies in the case of sleeping sickness) are absent, although for most vector-borne diseases, little is known about the capacity for alternate insects to become vectors.

U.S. Citizens at Risk for Tropical Diseases

The risks of contracting tropical diseases in the United States, either from infected individuals or from insects transmitting disease, are compounded for Americans who travel to other countries.

The longstanding interest of DOD in tropical diseases has practical roots. It is said that during World War II, more U.S. troops in the Philippines were hospitalized with malaria than were hospitalized with injuries of war. The same was true of our troops in Vietnam. Currently, more than half a million American military personnel are abroad, many located in the tropics (365).

In addition to military personnel, substantial numbers of Americans reside in less developed countries as employees of U.S. and international aid and development agencies or of multinational corporations. An even greater number annually travel to the tropics, no longer so far off and inaccessible as they once were. **In all, about 5 million Americans each year are at risk of contracting a tropical disease.**

APPROACHES TO CONTROLLING TROPICAL DISEASES

The goal of most tropical health programs is control of disease. In this report, the term control refers to the reduction of morbidity and mortality from disease using any or all of the spectrum of biomedical and environmental tools. Control is not synonymous with eradication, because eradication implies permanent elimination of a disease from the face of the earth. **While the possibility exists that certain tropical diseases can be eradicated, following the example of the spectacular success with smallpox, most of these diseases cannot be either because of biological or practical constraints.** Many tropical diseases can be *eliminated* from geographic areas, while existing in other parts of the world. The control of others can be achieved by maintaining low levels of incidence and prevalence.

Providing clean water and sanitation systems, coupled with education and behavioral changes, removes the sources of infection and the means of transmission for many diseases that are rife in developing countries. In the United States, at least since 1900, long before the antibiotic era, the spread of public works, particularly sanitation measures, was leading the country out of the thrall of infectious diseases. As Lewis Thomas has said, "Much of the credit [for improved health] should go to the plumbers and engineers of the Western World" (413).

The development of more specific disease control measures is heavily dependent on basic and applied biomedical research. The probability that researchers will develop successful control measures for tropical diseases has never been greater than it is today.

The advent and explosive growth in the use of "biotechnology"—recombinant DNA techniques and other sophisticated tools relying on the ability to harness and manipulate genetic material—have given a boost to the study of tropical diseases. Using methods formerly unavailable, immunologists and molecular biologists are beginning to understand the unique biology of the parasitic organisms that cause malaria, schistosomiasis, trypanosomiasis, filariasis, and leishmaniasis. This progress has led to vaccine research and to a new generation of badly needed diagnostic tools for parasitic diseases, as well as bacterial and viral diseases.

Although there is obviously great progress in controlling tropical diseases to be made through the use of biotechnology, there remains a need to continue more traditional research approaches in parasitology, infectious disease natural history, and basic biomedicine. This need is particularly acute for tropical diseases, because these diseases are much less well understood than diseases of importance in the United States. Such basic information about tropical diseases as the ranges of insect vectors that transmit many of them, their natural history, and their prevalence is incomplete. Although control of one or a few of these diseases is conceivable without such information, for most of the diseases, such information will be required before adequate control measures—which will of necessity integrate aspects of vector control, prevention, diagnosis, and treatment—can be designed.

The control of mosquitoes and other vectors (where appropriate) has been a successful route of disease control in certain geographic areas for several diseases. The elimination of malaria in the United States, in most of Europe, the Caribbean islands (except for Haiti and parts of the Dominican Republic) and other parts of Latin America was accomplished, at least in part, through control of the mosquitoes that transmit the disease. Similarly, through control of mosquito vectors, yellow fever was eliminated from Panama at the turn of the century, allowing completion of the Panama Canal.

Before the use of DDT (dichloro-diphenyl-trichloroethane) in the 1940s, vector control programs were based largely on physical measures, particularly on removing standing water where larvae could develop. In Africa, early control programs for tsetse flies, the vectors of African sleeping sickness in humans and nagana in livestock, relied on geographic isolation of flies (one strategy involved burning swathes of vegetation to prevent migration) and efforts to kill large numbers of flies manually. Although some schemes were more successful than others, the tsetse control effort on the whole, failed, at least in part because of a lack of effective coordination among a number of African nations.

After the advent of DDT, hope for controlling and eradicating diseases focused on chemical pesticides. The "malaria eradication program," announced in the mid-1950s, was WHO's first global initiative. WHO's promotion of a malaria eradication campaign was based on what is perceived in retrospect as the overly optimistic hope that a single intervention by insecticide could be effective. An appreciation of the ability of insect vectors to develop resistance to pesticides and of the complexity of interactions among vectors, humans, and the environment was the lesson learned from WHO's unsuccessful efforts. **Pesticides, even DDT, still have a place in strategies for disease control, but no single technology is likely to be able to control most vectors of disease.** The integration of chemical and biological approaches in "integrated pest management" (IPM) may provide the long-term solution, though as yet successes are few in this rather new field.

The application of biotechnology and immunologic tools has yielded a great deal of information about the biology of disease vectors. Specific arthropod vectors once thought to be homogeneous single species are now known to be "species complexes." Different species complexes may vary subtly or dramatically in their behavior and susceptibility to the organisms that cause disease. While appearing identical, their roles as disease vectors may be distinct as well. Knowledge about the vectors that transmit certain tropical diseases may become the basis for designing rational control programs.

As a technology, vaccination to prevent disease has had the greatest impact on health to date and still holds enormous potential. The principle of vaccination—the stimulation of the body's ability to fight off pathogens before they can cause disease—was established long before the germ theory of disease was developed. As early as 500 years ago, it was common practice in India, China, and probably Africa to scratch a bit of material from smallpox pustules into the skin of healthy people. The result of this process, called variolation, was to provoke a mild case of disease and thus render the person immune to further infection. Today's familiar vaccines—measles, rubella, mumps, diphtheria, whooping cough, tetanus, and yellow fever, for example—have been developed through one of several "conventional" methods. The pathogenic organism itself (attenuated or inactivated) or part of the organism is deliberately introduced into the body to prime the immune system to combat future infections.

Vaccine development has moved into a new era with biotechnology (361). Scientists' ability to decipher genetic codes and pinpoint the proteins that trigger an immune response has opened the door for subunit vaccines that may be safer and more specific than those derived from whole or partial disease organisms themselves. The first genetically engineered human vaccine, for hepatitis B, is now in early immunogenicity trials in humans. Progress toward a malaria vaccine puts initial trials in humans perhaps as early as 1986, but development of the vaccine for general use is probably 5 to 10 years off if no major problems occur.

Besides the development of the vaccines themselves, the mode of vaccine delivery occupies the attention of biomedical researchers and engineers. The "bifurcated needle," nothing more than a double-pronged needle which holds one drop of vaccine between the prongs, is one of the technologies credited with making smallpox eradication possible. The various vaccination devices used previously were, for a variety of reasons, not as well suited to the needs of the worldwide smallpox campaign. The bifurcated needle was developed by industry in response to the needs of the campaign, which was already under way.

Trials of "aerosolized vaccines," which are inhaled, have been going on for the past 10 years. Just last year, in 1984, Albert Sabin, the oral polio vaccine pioneer, reported successful measles immunization using an aerosolized vaccine. Delivered in an aerosol form, the vaccine was more effective than it had been when given by injection (301). This technique may prove important for a wide range of ARIs.

Just as important as vaccination devices in the tropics are technologies for the mass production, storage, preservation, and distribution of vaccines. Concerns about these functions relate to the stage of development of organized health services as well as to medical technologies per se. One of the most critical needs for immunization programs involving certain vaccines, for instance, is the maintenance of a "cold chain," the means to keep vaccines cool during transportation from the laboratory to the vaccinee, wherever that person might be.

Lack of adequate diagnostic technologies has hampered the study and treatment of many tropical diseases. In addition to benefiting individual patients, diagnostics are needed to learn about the ranges of diseases and their prevalence and incidence. **Although there is a wide range of conventional diagnostic tests that are adequate for some tropical diseases, the need for rapid tests that do not require sophisticated laboratory equipment remains.**

Biotechnology has made significant contributions in diagnostics. As a result, rapid diagnostic tests, which can be used under field conditions, are now under development. Diagnostic capability based on the DNA of the disease organisms has also brought to light within-species differences in disease organisms. Although the differences are subtle, they may eventually have significance for controlling diseases.

Research and development in therapeutic technologies for tropical diseases have lagged behind research and development in technologies for diseases of U.S. importance. **Few new drugs have been introduced for human tropical diseases in the past two decades, though there has been a surge in the development of products for parasitic infections of domestic animals. One reason why pharmaceutical companies have been reluctant to invest heavily in drugs for tropical diseases is that many of the potential beneficiaries of drugs cannot afford to buy them.** While a company may produce an effective drug, there may be no market for it.

Notwithstanding the general slow progress in the development of therapeutics for tropical diseases, there have also been some new and exciting developments.

The discovery in the 1960s that glucose is actively transported in water into the body through the intestinal wall even during severe diarrhea paved the way for the development of ORT. ORT is a major therapeutic measure for diarrheal dehydration that has the potential to significantly alter the mortality statistics of developing countries today. It is not a pharmaceutical in the usual sense, nor is it even a curative agent. ORT is a nonspecific treatment for episodes of diarrhea, particularly prevalent among children, and responsible for at least one-third of all infant deaths in developing countries.

ORT was developed as a treatment for cholera, first in adults, then in children and infants. Its efficacy for a wide spectrum of diarrheal diseases has since been proved. Increasingly, less developed countries are undertaking national diarrheal disease control programs in which ORT constitutes the keystone of the program. WHO and AID both are supporting major programs in ORT.

Praziquantel, a drug marketed in 1980, has revolutionized the treatment of schistosomiasis. Previously, schistosomiasis treatment itself involved major health risks, and was a long process. Praziquantel has overcome both of these problems and would probably replace the previous drugs of choice (which have disadvantages) if it were not so expensive.

Several new drugs for prophylaxis and treatment of malaria have been introduced in the past decade. The development and spread of malaria parasites resistant to chloroquine, for many years the drug of choice, has made the quest for new antimalarial drugs imperative.

Activity against a tropical disease by an antiviral drug, ribavirin, has been demonstrated for the first time. This broad spectrum antiviral was shown to be effective against Lassa fever in animals by DOD researchers. CDC scientists took ribavirin to the field in west Africa, where Lassa fever is a major problem, and the drug proved effective in humans.

STATUS OF CONTROL FOR SELECTED TROPICAL DISEASES

Control measures for tropical diseases vary in their availability, safety, and effectiveness. The status of preventive, diagnostic, and therapeutic technologies is summarized below for the diseases considered in this report.

Malaria

Malaria is one of the most widespread diseases in the world. In the last decade, its world prevalence has increased more than twofold (430). Worldwide, an estimated 250 to 300 million cases occur each year. In tropical Africa alone, an estimated 160 to 200 million people are infected every year, and 1 million people die, mostly infants and children. Human malaria is caused by four species of protozoan blood parasites of the genus *Plasmodium*. Different species are important in different parts of the world, although there is some overlap. The parasites have a complex life cycle and are transmitted to humans by mosquito vectors of the genus *Anopheles*.

The tools now available to control the mosquitoes that transmit malaria are inadequate to the task. Varying degrees of resistance have been developed by the mosquitoes to every insecticide that has been tried, and there is no reason to hope that a new conventional insecticide will be more successful on a long-term basis. Work on biological control methods (e.g., the introduction of mosquito predators) is needed.

Another problem in malaria control is the spread of strains of malaria parasites that are resistant to chloroquine and other antimalarial drugs. Screening and testing of new compounds has proceeded at a steady pace since about World War II, however, and a few of new drugs are now in various stages of development and testing.

The most exciting development in malaria control is the prospect of a vaccine. Advances in biotechnology have made hopes for a vaccine realistic. With continued success, optimistic estimates put a malaria vaccine on the market in 5 to 10 years (see case study B).

Schistosomiasis

Schistosomiasis is a chronic, debilitating, parasitic disease caused by trematode worms of the genus *Schistosoma* that live in vertebrate host blood vessels. The three major species of schistosomes that affect humans (*S. mansoni, S. haematobium*, and *S. japonicum*) have a complex life cycle, requiring certain freshwater snails as intermediate hosts. These parasites together have a pantropical distribution. A 1972 survey including 71 countries estimated that 500 million people were exposed to schistosomiasis and 125 million were infected.

In recent years, there has been a drop in the incidence and prevalence of schistosomiasis in some areas and an increase in others. Preventing transmission of schistosomiasis is accomplished most effectively by reducing human contact with infected water. Piped water supplies and excreta disposal are therefore the most effective control measures. Molluscicides (agents for killing snails) are available, but all have adverse environmental side effects and do not provide a permanent solution to control. Biological methods to control snails are under investigation.

In some areas, large-scale hydroelectric and agricultural irrigation projects have been responsible for spreading schistosomiasis by providing year-round breeding sites for snails. The best known case is the Aswan High Dam in Egypt. According to a 1937 survey, about 5 percent of the population in upper and middle Egypt was infected with schistosomiasis. Following the construction of the Aswan High Dam, the prevalence rate rose to 30 percent. Areas where large-scale development projects are undertaken may require special surveillance and control measures to prevent human health problems.

Chemotherapy for schistosomiasis, using Praziquantel, along with previously available drugs, is effective and relatively safe. Work on a schistosomiasis vaccine is still in exploratory stages.

Trypanosomiasis

Trypanosomiasis is a group of clinically different diseases of the blood and tissues caused by different species of the genus *Trypanosoma*. The important human diseases are African sleeping sickness and Chagas' disease.

African Sleeping Sickness (African Trypanosomiasis)

African sleeping sickness is caused by two varieties of *Trypanosoma brucei*, which are transmitted by different species of tsetse flies (genus *Glossina*). In west Africa, the disease is caused by *T. brucei gambiense*, while in east Africa, it is caused by *T. b. rhodesiense*. *T. b. gambiense* causes a chronic, debilitating disease in humans that saps the energy and eventually kills. *T. b. rhodesiense* infects both humans and animals. People become infected when they enter hunting grounds and grazing areas where there are infected animals. The disease is rapidly fatal to humans and destructive to domestic livestock.

Control of tsetse fly vectors of sleeping sickness has, by and large, been unsuccessful. Consequently, large areas of land have been abandoned or left unsettled because of the threat of disease.

Therapy for African sleeping sickness requires hospitalization because of the use of toxic, intravenously administered, and frequently only partially effective drugs over an extended period of time. Safe, effective, short course chemotherapy is needed.

Chagas' Disease (American Trypanosomiasis)

Chagas' disease occurs in almost every country of Latin America. The disease is caused by *Trypanosoma cruzi*, a protozoan parasite that lives in the blood and tissues. It is transmitted to humans and about 150 other species of mammals by reduviid bugs, blood-sucking insects found throughout the Americas. Reduviid bugs are harbored in the mud and thatch of substandard housing, and the transmission of Chagas' disease is especially high in rural areas. In 1974, WHO estimated that out of 50 million exposed, 12 million people were infected with *T. cruzi*.

The acute phase of Chagas' disease, which may cause heart and nervous system damage, can be fatal, but it usually passes into a chronic stage. Heart failure and grotesque enlargement of the digestive tract are among the long-term sequelae which lead to death.

There is no effective treatment beyond the acute phase and no vaccine. Control measures for Chagas' disease concentrate on periodic insecticide spraying of houses. Improved house construction, which could eliminate breeding sites for aduviid bugs, could provide a permanent control solution.

Leishmaniasis

Leishmaniasis is the collective term for a spectrum of parasitic diseases caused by several species of the protozoan genus *Leishmania*. All species, which segregate largely into Old World and New World forms, are transmitted by blood-sucking phlebotomine sandflies. In 1977, an estimated 400,000 new cases of leishmaniasis occurred worldwide, and in some countries, the number is increasing. Many wild animals in jungle areas that are being cleared for agricultural development are infected with leishmanial parasites, putting new settlers at risk.

Depending on the infecting species, leishmaniasis takes several clinical forms. In the least severe form, cutaneous leishmaniasis, self-resolving skin lesions appear at the site of the insect bite. In the mucocutaneous form, sores may spread into the nasal and pharyngeal mucous membranes, disfiguring the face, nose, and throat. The most destructive form, called "kala azar," attacks internal organs—spleen, liver, bone marrow, and lymph glands—and in epidemics kills thousands of people. Recent epidemics in Asia have resulted from reemergence of the sandfly vectors after spray programs were discontinued.

There is no vaccine against leishmaniasis. Therapeutic drugs for treating leishmaniasis, mainly antimony compounds, are not always effective and have serious side effects. Rapid field diagnostic techniques are being developed through biotechnology. These techniques could both help patients by detecting disease earlier in its course and facilitate field epidemiologic studies of the natural history of the disease. Overall, control of leishmaniasis is poor.

Filariasis

At least eight species, in several genera, of filarial nematode worms, threadlike in form, inhabit the skin, other tissues, or the lymphatic system, causing filariasis in humans. These parasites are transmitted by blood-sucking insects.

Of the many filarial worms that infect humans, three are of major public health importance on a global scale: *Wuchereria bancrofti* and *Brugia malayi*, both of which can result in elephantiasis; and *Onchocerca volvulus*, which is the agent of onchocerciasis (river blindness). *W. bancrofti* and *O. volvulus* are widespread in Africa, and *B. malayi* in parts of Southeast Asia. All three of these species have become established to lesser degrees in the New World.

There are no vaccines to prevent filariasis. Treatment for all forms of filariasis is similar. There is effective therapy for the microfilariae that cause the symptoms of disease, but no nontoxic therapy against adult worms. All available drugs have side effects, some serious. There is a great need for improved filaricidal agents and immunopotentiating agents, drugs able to stimulate the natural defenses of the body.

Leprosy (Hansen's Disease)

Worldwide, an estimated 15 million people have leprosy, most of them in the Old World. The disease ranges from "tuberculoid" leprosy, with localized skin lesions and minor nerve involvement, to the severe "lepromatous" leprosy, with spreading, disfiguring lesions, resulting in destruction of the nose, involvement of the vocal cords and eyes, and severe nerve damage.

The agent that causes leprosy, *Mycobacterium leprae*, is closely related to the bacterium that causes tuberculosis. Although leprosy has been recognized and feared for centuries, frustratingly little is known of its natural history. Even the way in which leprosy is transmitted is not clear. The most likely means is through the respiratory tract, infection being acquired by direct contact with infected individuals.

Treatment of leprosy involves months or years to a lifetime of treatment with a combination of drugs. There are only a handful of useful drugs, most of which do not actually kill the bacteria, but only arrest its spread. Resistance has developed even to the most effective drug. Considerable progress has been made toward a leprosy vaccine, but it is difficult to predict whether a vaccine will be successful as a control measure for the majority of those at risk. Technical problems in studying leprosy, particularly scientists' limited ability to culture *M. leprae* in the laboratory, have hampered research in all areas of leprosy control.

Tuberculosis

Tuberculosis is a major public health problem in most developing countries and is a resurgent problem in some crowded, poor inner cities in the United States. It is also the most common serious infectious disease among Indochinese refugees to the United States. Tuberculosis is caused by the bacterium *Mycobacterium tuberculosis* and is transmitted from person to person by airborne droplets. The lungs are infected first, but infection can subsequently spread to all parts of the body. Infections are chronic and debilitating if untreated.

The vaccine against tuberculosis is known as BCG (Bacillus Calmette-Guerin) vaccine. BCG vaccine is used mainly in areas of high transmission rates, but its effectiveness has shown great variability in field trials in different parts of the world. There is adequate treatment now for most cases of tuberculosis, though there are drug-resistant strains. A minimum of 6 to 9 months treatment is necessary with the best drugs in combination. In many tropical countries that use less expensive and less effective drugs, much longer treatment is necessary. New antibacterial agents are needed.

Diarrheal and Enteric Diseases

Diarrheal diseases occur throughout the world. Research since the early 1970s has uncovered an array of organisms that cause diarrheal diseases, and there is a continuing need to identify and characterize these organisms. The agents of diarrheal diseases include viruses, bacteria, and protozoa. These agents are transmitted to humans by fecal contamination of food or water.

The greatest danger in diarrheal disease, regardless of the specific cause, is severe, life-threatening dehydration and shock. In adults, diarrheal diseases other than cholera are generally not life-threatening. In children under 5 in many developing countries, however, diarrheal diseases are the leading cause of illness and death. Infants and young children in developing countries may experience four to eight separate episodes of diarrhea per year. One out of every 150 to 200 episodes results in severe, life-threatening dehydration.

One way to prevent diarrheal diseases is to break the transmission cycle by providing clean water and sewage disposal. The encouragement of breast feeding, proper weaning practices, and health education could also help. Vaccines against rotaviruses, *Shigella*, and *Salmonella*, the agents of a large percentage of childhood diarrhea, are undergoing development.

From World War II through the 1970s, the only accepted means of treating diarrheal dehydration was through intravenous replacement of body water and salts. In the late 1960s and continuing into the 1970s, a therapy for diarrheal dehydration was developed that has been called the most important therapeutic advance in tropical medicine. That therapy, ORT, involves the oral administration of a simple solution of water, salt, and sugar and thus does not require hospital facilities (see case study B). ORT has revolutionized the treatment of adults with cholera and, even more importantly, is effective in infants and children with a wide range of diarrheal infections.

Acute Respiratory Infections (ARIs)

ARIs are among the most important causes of preventable deaths in the world. These infections include both lower respiratory tract infections (e.g., pneumonia and bronchitis) and upper respiratory tract infections (e.g., influenza, measles, diphtheria, and whooping cough). The organisms that cause ARIs are transmitted by airborne droplets and include viruses, bacteria, and mycoplasma.

In developed countries, the toll from ARIs (other than influenza in pandemic years) is relatively low and stable. ARIs are exacerbated by malnutrition and substandard living conditions, however, and in developing countries, these infections take a large annual toll. Some 12 percent of all deaths of children living in Africa, Central America, and Asia are attributed to ARIs. ARIs not only pose a serious mortality risk for the very young and very old in developing countries, but reduce productivity in all age groups and impose tremendous demands on health care systems.

Effective vaccines are available for measles, pertussis (whooping cough), and diphtheria, but a large percentage of the world's children have not been vaccinated. Immunization rates have increased in some countries as a result of WHO's Expanded Program on Immunization (EPI), but EPI's goal of universal vaccination of all children is still far from being realized. Vaccines for pneumococcal pneumonia and influenza are available, but have limited use in developing countries.

The lack of adequate rapid diagnostic methods that can be used in less developed areas of the world hampers effective treatment of ARIs. Some researchers are attempting to develop new diagnostics with the methods of biotechnology. Effective treatments exist for many of the bacterial and mycoplasmal ARIs, but the development of antiviral chemotherapy is still in its infancy. For bacterial ARIs, drug resistance to penicillin and other antimicrobials is an increasing problem.

Arboviral and Related Viral Infections

The name "arbovirus" is derived from the descriptor "arthropod-borne virus." The unifying characteristic of arboviruses is that they replicate in and are transmitted by arthropods (predominantly mosquitoes, but also ticks, sandflies, midges, and gnats). Arboviruses occur worldwide, infecting humans and other animals. The 80 or so human types have varying distributions and exhibit an apparent trend toward spread rather than containment. Many of the viruses that principally infect animal populations also cause occasional severe outbreaks in human populations.

Important arboviral infections in terms of severity and prevalence are yellow fever, dengue fever, oropouche fever, chikungunya, Japanese encephalitis, other viral encephalitides, and hemorrhagic fevers. Clinically, arbovirus infection can cause either mild disease (e.g., acute benign fevers or self-limiting arthritic symptoms), severe central nervous system disease with brain inflammation which can be fatal, or hemorrhagic fevers with high case-fatality rates. Yellow fever also causes liver damage and jaundice.

Effective vaccines exist for yellow fever and Japanese encephalitis, and work is proceeding on vaccines for dengue fever and a few others. Vector control, at present, provides the most promising avenue for control. Development of therapeutic drugs for arboviral infections is still in its infancy. Treatment for such infections now consists mainly of relieving symptoms.

U.S. EFFORTS IN TROPICAL DISEASE RESEARCH

Rationale for U.S. Efforts in Tropical Disease Research

The reasons for U.S. involvement in tropical disease research, and in the whole area of international health, have not changed since the end of World War II. It was at that time that the United States began to provide substantial amounts of aid to encourage the development of less developed nations. The Interdepartmental Committee on International Health Policy, a group set up in 1960 as one attempt at coordination among the interested agencies, identified four basic objectives of international health policy. Restated here, they are (75):

1. **Social and humanitarian objectives.** The ideals and the humanitarian and philosophical beliefs basic to the American democratic tradition should be reflected in U.S. international health activities. The desire for the world's people to enjoy good health is an extension of the attitude of Americans toward their own health.
2. **Political objectives.** Involvement in international health contributes to the U.S. position of world leadership. The United States is not alone in supplying aid in international health. In addition to European nations, Cuba, for instance, is extremely active, with health personnel now in about 25 countries (348).

Health programs can contribute to the political stability of developing nations.

3. **Economic objectives.** Improved health in developing countries can have economic benefits for those countries as well as for the United States. For the countries themselves, increased productivity can accompany gains in health. For the United States, markets for U.S. goods and the atmosphere for U.S. investments may improve with increased involvement in the health sector.
4. **Medical objectives, including self-protection against diseases.** The self-interest of protecting Americans from tropical diseases is increasing in importance as the world community grows smaller. Americans abroad, as well as at home, may be exposed to the risks of tropical diseases. In addition, there may be spinoffs in understanding or controlling diseases of importance in the United States. For example, techniques developed during the campaign against smallpox have been successfully applied to measles control in this country (161).

To these four may be added a fifth objective:

5. **Scientific objectives.** Tropical diseases pose challenging problems for scientists who are interested in advancing knowledge and improving health.

U.S. Activities in Tropical Disease Research

The major U.S. Government supporters of tropical disease research are: NIH, mainly the National Institute of Allergy and Infectious Diseases (NIAID), and CDC (both in DHHS); DOD; and AID. Each U.S. Government agency supporting tropical disease research acts in accordance with its own specific mandates, which by and large do not overlap with the mandates of other agencies. NIH funds research of scientific merit that will advance the state of knowledge about causative agents, the response of humans to the disease agent, and the disease itself. CDC responds to specific problems around the world in the control of diseases as they affect the health of U.S. citizens, and also maintains applied research programs in diseases of public health concern (e.g., malaria and helminthic diseases) (27). DOD focuses on health problems of direct relevance to U.S. military personnel in different parts of the world. Most AID-funded research has relevance to development assistance and political objectives.

The Federal agencies that fund research do so under various legislative mandates, which for the most part allow, but do not necessarily encourage, delving into tropical health issues. To quote a 1978 report of the White House Office of Science and Technology Policy (27):

> At best, current authorization is passive and certainly does not act as a stimulus. Other legal restrictions further limit the use of agency authorizations to support international health research.

The situation has not changed appreciably since that report was issued.

The U.S. Government also contributes to research through programs supported by WHO and through TDR (the Special Program for Research and Training in Tropical Diseases) which is administered by WHO, cosponsored by the U.N. Development Program and the World Bank, and funded by international contributions).

Private sector supporters of tropical disease research include foundations, pharmaceutical companies, volunteer agencies, and religious organizations. Private U.S. foundations have programs that have often served to highlight problems and identify opportunities not exploited by Federal agencies. The Rockefeller Foundation's Great Neglected Diseases program and the Edna McConnell Clark Foundation's focus on schistosomiasis are examples.

U.S. Funding of Tropical Disease Research

It is difficult to pin down a dollar figure that represents U.S. research in tropical diseases, because information is not standardized across agencies, and definitions of tropical diseases differ. **On the basis of analyses of information developed for this assessment, OTA estimates that, in recent years, the U.S. Government has annually contributed less than $100 million toward research in tropical diseases, out of a total annual biomedi-**

cal research budget of well over $4 billion. Contributions of private foundations are of lesser magnitude. Figures on tropical disease research funding by pharmaceutical companies are undocumented.

U.S. Department of Health and Human Services

NIAID's tropical medicine funding covers research in three general areas: 1) traditional tropical diseases (malaria, schistosomiasis, trypanosomiasis, leishmaniasis, filariasis, and leprosy); 2) general parasitology (cestodes, nematodes, trematodes, and protozoa); and 3) general tropical medicine (rickettsia, bacteriology, mycology, virology, and vector pathogens). In fiscal year 1983, NIAID funding for tropical medicine research was about $33 million, about 12 percent of NIAID's budget (less than 1 percent of the total NIH budget). About one-quarter of this funding supported researchers working at NIH (intramural research), and three-quarters went to researchers outside NIH (extramural research). In addition to the $33 million for tropical medicine research, NIAID spent approximately $3 million on projects in diarrheal diseases and ARIs (a large proportion of ARI funding is for influenza research, directed at domestic problems).

The Fogarty International Center of NIH does not have a tropical disease research program, but approximately $2 million annually is channeled through the Fogarty budget to the Gorgas Memorial Institute, for the operation of its laboratory in Panama. Fogarty also holds international conferences and provides fellowships to foreign scientists, some of which contribute to the overall effort in tropical disease research.

CDC conducts tropical disease research primarily through the Center for Infectious Diseases and its Division of Parasitic Diseases. In fiscal year 1983, CDC spent an estimated $5 million on tropical disease research. This includes support of CDC's Medical Entomology Research and Training Unit in Guatemala.

U.S. Agency for International Development

Most AID funding for tropical disease research comes from the Office of Health. In fiscal year 1982, this amounted to about $14 million. About $5 million of this was the U.S. contribution to TDR, about $5.8 million was spent on research in malaria immunology and vaccine development, and $1.9 million was part of the core support for the International Center for Diarrheal Disease Research in Bangladesh. AID's Africa Bureau spent approximately $1.3 million on the biomedical research components of three projects: onchocerciasis control, combatting communicable childhood diseases, and schistosomiasis activities in Cameroon and the Sudan.

AID's Office of the Science Advisor supports additional research at the level of about $1 million in grants through the Program in Science and Technology Cooperation (PSTC) and through the National Research Council's Board on Science and Technology for International Development (BOSTID). Most of these funds go to researchers in developing countries.

U.S. Department of Defense

In fiscal year 1982, DOD spent about $13.5 million for biomedical research in tropical infectious diseases, representing about 6 percent of all DOD biomedical research funding. The Army and Navy conduct a certain amount of research themselves, as well as contracting out research both in the United States and in the tropics. The U.S. Army currently maintains the only large-scale antimalarial drug screening program in the world. It also maintains medical research units in eight developing countries, the largest such program of any U.S. Government agency.

U.S. Contributions to International Programs

The **Special Program for Research and Training in Tropical Diseases (TDR)**, administered by WHO, is cosponsored by WHO, the World Bank, and the U.N. Development Program. TDR was initiated in 1975 and has two main objectives: 1) to strengthen the biomedical research capabilities of the developing countries; and 2) to develop tools to control six tropical diseases (malaria, schistosomiasis, trypanosomiasis, leishmaniasis, filariasis, and leprosy).

By the end of 1981, TDR had received more than $95 million in contributions from 25 governments, other organizations, and the sponsoring agencies. The U.S. annual contribution to TDR was $4 million in 1980 and 1981 and just over $5 million in 1982.

WHO supports some tropical disease research in addition to its contributions to TDR. Under the general classification of communicable disease prevention and control, about $2.4 million of WHO funds went for research in tropical diseases during the 2-year period 1980-81.

WHO also supports two separate programs relevant to tropical diseases: one in ARIs and one in diarrheal diseases. Over the 2-year period 1980-81, WHO spent $381,000 for ARI research and just over $1 million for diarrheal disease research. WHO's 1982-83 diarrheal disease research budget was estimated to be about $3.8 million.

Precisely determining the U.S. contribution to WHO research programs is difficult, because funding for each set of projects comes both from the "regular budget" and from a spectrum of "extrabudgetary sources," in varying amounts. For the period 1980-81, the annual U.S. monetary contribution is estimated by OTA to be between $250,000 and $500,000.

Funding of U.S. Researchers in Tropical Diseases

Most of the tropical disease research funded by U.S. institutions is carried out by U.S. researchers. In fiscal year 1982, for example, NIAID awarded 96 percent of its tropical medicine funds to U.S. institutions and 4 percent to researchers in other industrialized countries. Often, however, investigations undertaken by U.S. institutions are carried out in close collaboration with institutions in less developed countries.

AID's Bureau of Science and Technology allocates almost 75 percent of its biomedical research funds to American institutions. Many of these institutions collaborate with organizations in the less developed countries. AID's Research Grants Program, administered by the Science Advisor's Office and by BOSTID (NAS), has so far allocated about 45 percent of its resources to American institutions, though the BOSTID portion of the program makes grants only to institutions in developing countries.

The DOD biomedical research program allocates about 75 percent of its extramural funds to organizations in the United States, 18 percent to institutions in less developed countries, and 5 percent to organizations in other industrialized countries.

The majority of biomedical research funds both of the Rockefeller Foundation (52 percent) and of the Edna McConnell Clark Foundation (77 percent) were awarded to American institutions.

U.S. researchers also receive funds from international organizations in excess of the U.S. contributions to such organizations' research budgets. In 1981, TDR awarded about one-third of its research funds to Americans, nearly equal to the U.S. contribution. The same year, WHO's program in diarrheal diseases awarded one-quarter of its research funds to American institutions, but the U.S. contribution represented 1 percent of the program's total budget.

OPTIONS

Development of Medical Technologies for Tropical Diseases

To encourage research and development of medical technologies for tropical diseases, Congress might consider the following options.

OPTION 1: Explicitly include drugs and vaccines for tropical diseases in the definition of "orphan drugs" under the Orphan Drug Act of 1983 (Public Law 97-414).

OPTION 2: Encourage Federal agencies, such as AID, to examine the possibility of interesting private companies in developing medical technologies for tropical diseases by guaranteeing purchases of products and assisting in field trials.

OPTION 3: Mandate the creation of and authorize funds for a quasi-governmental nonprofit corporation to undertake research and development of medical technologies for tropical diseases until the technologies become economically attrac-

tive enough for private industry to take over with the right to an exclusive license for the product.

OR

Stimulate the development of an international nonprofit corporation, funded through contributions from the U.S. Government, other governments, and international bodies, to undertake such research and development.

OR

Create a nonprofit corporation charged with ensuring the development and availability of prophylactic and therapeutic agents for use in developing countries for which there appears to be insufficient commercial interest.

Relatively few U.S. pharmaceutical companies are pursuing the development of drugs and vaccines for tropical diseases. The few that are often have entered the field because research for tropical parasitic diseases of humans is a spinoff of research on parasitic diseases of domestic agricultural animals. There is, at present, little financial incentive for pharmaceutical company activity in most human tropical diseases. The 1978 Office of Science and Technology Policy report states (27):

> There is almost no domestic U.S. market for vaccines, drugs, or pesticides used against tropical diseases. The main potential purchasers of these products are developing countries or international assistance organizations acting on their behalf. At present, these markets are unprofitably small and offer no realistic incentive for industry research in this area. . . .
>
> We conclude that underutilization of existing drugs and vaccines, researched and developed at considerable expense by industry, is a major disincentive to new investment in tropical medicine research and development by pharmaceutical firms.

It is unfortunate that financial incentives are lacking to develop drugs for some of the most widespread diseases of humankind. The lack of profitable markets does not correlate with a lack of patients, but with the high price of newly developed drugs (necessary to recoup research and development costs); the relatively small health budgets of developing country governments and the inability of most people in developing countries to pay for the drugs themselves; and unstable political conditions which make it risky for U.S. companies to rely on trade with specific developing countries.

The new medical products developed with biotechnology, largely vaccines and diagnostics, might provide a stimulus for activity by the pharmaceutical industry. With much of the developmental work on these products coming from publicly funded research, the research and development costs incurred by industry should be somewhat less. Much of the expense of production, particularly for vaccines, however, is incurred in scaling up production and in clinical trials.

As the malaria vaccine now under development in the United States illustrates, there are also issues to be addressed in the commercialization of publicly funded research. Funding for the malaria vaccine's development has come from various U.S. Government agencies, private foundations, and WHO. WHO's policy is that the fruits of WHO research funding should be available to the world at large. This policy means that a commercial company desiring to produce the malaria vaccine, should it move to the production stage, would not be allowed to secure a patent. Because substantial costs are involved in developing a process for large-scale production of the vaccine, many companies would be unlikely to undertake the project without patent protection.

The Orphan Drug Act of 1983 charges the U.S. Government with the task of identifying and promoting "orphan products," defined as drugs and devices for rare diseases or conditions. The act defines rare diseases and conditions as ones that occur so infrequently that there is no reasonable expectation that the cost of development can be recouped by sales within the United States.

Four kinds of support for orphan drugs are authorized by the 1983 act:

1. A 50-percent tax credit on all clinical testing expenses associated with the drug.
2. Award of an exclusive 7-year right to market a drug that is unpatentable (through the Food and Drug Administration's new drug approval authority).

3. Technical assistance in the development of clinical testing protocols.
4. Award of grants and contracts for clinical testing expenses associated with an orphan drug.

In addition, public relations benefits may accrue to pharmaceutical companies developing orphan products.

The Orphan Drug Act is aimed at diseases that are rare in the United States, certainly the case with most tropical diseases. It does not address the prevalence of diseases in other parts of the world. The Food and Drug Administration (FDA) is planning to publish for public comment proposed regulations under the act. None now exist, though FDA is operating under interim guidelines and is designating orphan products. The regulations, when final, could encourage research and development in drugs for tropical diseases.

Even with the incentives of the Orphan Drug Act, there is little reason to expect a major increase in the activities of U.S. pharmaceutical companies in tropical disease research. One alternative is some form of public funding expressly for pharmaceuticals for tropical diseases. Although setting up a nonprofit corporation is not the only means of injecting public funds into the process, there might be advantages to setting up a corporation, rather than simply adding money to TDR or increasing grants through NIH. The nonprofit corporation's research could be expressly aimed at eventual commercialization, and rules could be set up for the transfer of projects to for-profit pharmaceutical companies at the appropriate time.

Information for Congressional Decisions

OPTION 4: Hold a special appropriations hearing for tropical disease research with representatives from NIH, CDC, DOD, and AID, and perhaps invite international agencies and private foundations to participate.

AND/OR

OPTION 5: Require each agency mentioned above to submit a short report on the status of its tropical disease research, providing data specified by the Appropriations Committees for use during appropriations hearings.

Evaluating the adequacy of U.S. research efforts in the area of tropical diseases is difficult, because no specific goals have been enunciated except in the case of U.S. contributions to the TDR and WHO programs. By and large, each U.S. Government agency involved in funding or carrying out tropical disease research does so according to its own needs, and, in most cases, through legislation that passively allows tropical disease research activities rather than promotes them.

In the past, suggestions have been made to improve coordination among the Federal agencies involved with tropical disease research (27,252). Two very basic aims of such coordination would be: 1) to avoid duplication of effort, and 2) to identify gaps in the overall research agenda.

OTA found no evidence that the tropical disease research efforts supported by different Federal agencies are duplicative. The professional community in international health is relatively small and closeknit, and within that community, there is a high degree of awareness of the various activities taking place. Undetected duplication of effort is relatively unlikely also because the total amount of money spent on tropical disease research is small.

There do appear to be gaps in current tropical disease research activities, however, and some of them are identified in this assessment. It is possible that a coordinating committee of some sort, with representatives from the relevant agencies, could identify gaps and devise plans to fill them. However, the options cited above would allow the Appropriations Committees to see the range of related activities in tropical disease research and might serve as well as or better than a coordinating committee.

Looking at just one component of tropical disease research does not allow an assessment of the adequacy of the effort. A look at the mix of Federal funding for malaria research, the most heavily funded disease-specific research, illustrates the value of examining information across agencies.

Of the funds NIAID spends for research on the six TDR tropical diseases, about 20 percent goes to malaria research. (The highest percentage of those NIAID funds, about 25 percent, goes to trypanosomiasis research.) The focus of NIAID's malaria work is on immunology and vaccine work. Of the money CDC spends for research on the six TDR diseases, 60 percent goes for malaria research. CDC maintains a colony of monkeys, large colonies of mosquitoes, and laboratory facilities for studies of malaria transmission, drug testing, and other biological characterizations. DOD is heavily involved in the screening and testing of potential antimalaria drugs. Most of the drugs currently available for chemoprophylaxis and treatment of malaria either have been developed by DOD researchers or their contractors or have benefited from DOD research. About 60 percent of DOD's budget for the TDR diseases goes for malaria research. AID's research activities in tropical diseases have focused on malaria immunology and vaccine development.

TDR itself allocates about 30 percent of its annual budget for malaria research. Most of TDR's funded projects concern immunology, including vaccine development, and chemotherapy of malaria. More than one-third of the WHO budget for parasitic disease research is spent on malaria.

To obtain basic information comparable across Federal agencies, the Appropriations Committees could provide a framework for the presentation of information at hearings or in a report from agencies.

The following types of information might be included in the reports from U.S. Government agencies:

1. A summary of funded activities for each disease from a core list of tropical diseases (e.g., the diseases covered in this report). The information listed below might be required, including, where appropriate, indications of which research is strictly associated with tropical diseases and which only partially:
   —type of research—basic or technology-oriented;
   —if basic, category (immunologic, physiologic, etc.); and
   —if technology-oriented, category (preventive, diagnostic, therapeutic).
2. The same information as in (1) for other diseases at the discretion of each agency:
   —number of projects; and
   —total funding levels.
3. A rationale for the allocation of funds.
4. An indication of how much research is funded at institutions in the tropics, and how much in the United States, and acknowledgment of funded American researchers collaborating with researchers in the tropics.
5. An indication of U.S. contributions to international research efforts (principally WHO and TDR).
6. An assessment of gaps in research as perceived by the agencies.

Private foundations and international agencies funding tropical disease research could provide similar information about their activities and could provide additional perspective on research needs.

Even given specific categories, Federal agencies will not be able to clearly identify all research directed at tropical diseases. There are two basic reasons.

First, some research will address health problems that are important both domestically and in developing countries. A clear example is research on ARIs, which are prevalent all over the world. Most of the ARI research funded by U.S. institutions is directed at the U.S. problem, particularly influenza, but progress may have spinoffs for the tropics.

ARIs are caused by a range of diverse and unrelated organisms, and one important research problem is to gauge the importance of different causal agents. The spectrum of ARI agents in the United States is probably quite different from that in the tropics, in which case, advances in diagnosis and treatment may benefit the United States without having an appreciable effect on conditions in developing countries. Some ARI research, however, is directed specifically at conditions in the tropics. In examining funding for tropical diseases, either including or excluding all general ARI research would be misleading. In cases such as ARI, the agencies could be asked to submit total funding amounts and estimates of the proportion related to conditions in the tropics, with a narrative describing how the breakdown was made.

The second reason for the difficulty in assigning research to the tropical disease category is that many tropical disease parasites, trypanosomes for instance, have become subjects of heuristic study in basic science laboratories. Because these parasites are interesting organisms, researchers have begun using them to study basic functions, such as the transport of substances across membranes and, in many cases, basic immunology, gene transcription, and gene regulation. These studies are not necessarily directed toward the control of disease and may be successful without ever contributing to a diagnostic, preventive, or therapeutic technology.

Research Funding

OPTION 6: Increase Federal funding for all aspects of tropical disease research.

OPTION 7: Amend the international health mandate of the U.S. Department of Health and Human Services (DHHS) to remove the limitations on the research DHHS may support in tropical diseases.

U.S. Government funding for tropical disease research in recent years has been less than $100 million annually, out of an annual Federal biomedical research budget of $4 to $5 billion. It is understandable that the disease problems of importance in the United States consume most of the resources, but there are perhaps a billion people in other countries suffering from or at risk for diseases which most U.S. citizens will never encounter.

Since the focus of this assessment has been tropical disease research itself, OTA has not identified specific funding programs or institutional bases for increases in funding. An NAS study now in progress is developing information that will be of direct value in formulating such programs and identifying the institutional resources that exist in this country. In the short term, however, it appears that the funding mechanisms that now exist could productively absorb funding increases.

Current authority for international health research within DHHS derives primarily from the International Health Research Act of 1960 (Public Law 86-610), the purpose of which is "to advance the status of the health sciences in the United States and thereby the health of the American people through cooperative endeavors with other countries in health research and research training." The authority to undertake research for the good of people outside the United States rests with the President.

Congress could transfer the authority to make decisions about all aspects of tropical disease research to DHHS itself, the Department which includes NIH and CDC. It could do this by amending the language of the International Health Research Act to give DHHS the authority to "advance the status of the health sciences in the United States and thereby the health of *all* people."

Such a change would explicitly provide greater justification for DHHS research on diseases that are not major public health problems in the United States. Giving NIH, in particular, more flexibility to fund research on tropical diseases could stimulate the submission of a greater range of grant proposals and might provide the basis for additional contracts in the area of tropical diseases.

2.

The United States in International Health

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2.
The United States in International Health

INTRODUCTION

Over the course of the 20th century, the U.S. Government and U.S. scientists and medical workers have sustained an interest in the health of people in other parts of the world. This chapter describes the reasons for U.S. participation in international health activities, and then discusses some of the important influences on those activities.

RATIONALE FOR U.S. INVOLVEMENT

In 1983, the United States spent more than $14 billion in foreign aid to less developed countries. Although much of this aid went for defense, the United States increasingly has recognized the value of assisting developing countries with health services and building health infrastructure. The health budget of the U.S. Agency for International Development (AID) for 1983 was about $123 million.

Five basic objectives of U.S. activities in international health were discussed in chapter 1. In general, the reasons for U.S. involvement in international health activities apply to the subset of tropical disease research. Briefly, the motives for U.S. involvement are:

- social and humanitarian;
- political;
- economic;
- medical, including protection of U.S. citizens against disease; and
- scientific.

The social and humanitarian motive is either convincing or not to an individual or a society, and is something that can shift in importance over time.

The medical motive, particularly to protect U.S. citizens, may be gaining strength, as it is becoming more apparent that opportunities for worldwide disease transmission have been and are continuing to increase. A still small but growing number of U.S. citizens are exposed to tropical diseases both abroad and at home (see ch. 1). Furthermore, the opportunities for worldwide transmission of disease have recently been demonstrated by the emergence of acquired immunodeficiency syndrome (AIDS) as a public health problem in the United States, particularly among the gay community. There is convincing evidence that the syndrome originated in Africa and is present in the heterosexual community there. Its appearance in the United States probably came through migrations of Africans to Haiti, where vacationing Americans came in contact with it (362).

The political, economic, and scientific objectives of U.S. involvement in international health activities are discussed further below.

Political Objectives

One aim of all U.S. international aid is to win friends for the United States, and thus sustain the U.S. role as a world leader. Contributions in health, particularly toward primary health care, may also be one paving stone on the road to social harmony and political stability. Furthermore, there is an opportunity to raise grassroots support for the United States by having Americans participate at local levels in developing countries.

The United States is not the only country providing health aid. One of the more active countries in recent years has been Cuba. Cuban medical personnel are present in more than 25 countries (348). Certainly in relation to size, Cuba accords tropical medicine much greater importance than does the United States. Cuba has virtually eliminated its classical tropical disease problems, so its present emphasis is not mainly a response to domestic needs, though surveillance to prevent reintroductions of once prevalent diseases is a priority.

In the summer of 1983, when issues concerning continued funding of the Gorgas Memorial Laboratory in Panama were addressed by OTA and the General Accounting Office, the role of Cuba was raised by an official of the U.S. State Department. It was feared that "closure of the Laboratory would give Cuba the opportunity to attract undue attention to its institute [the Cuban Institute of Tropical Medicine] which could be disadvantageous to the United States" (355). Going beyond the specific case of Gorgas, countries may be inclined to accept health aid from those best equipped and most willing to provide it.

Economic Objectives

There is intuitive appeal to the notion that improvement in the health of a population will lead to economic improvement. Solid proof of this intuition has been elusive, but some evidence now exists to support it. Belief in the concept that "health pays" is reflected in the actions of the World Bank over the past years.

The World Bank is [illegible]itution which, like commercial banks, req[illegible]hat projects for which loans are given be economically viable. The Bank first began making loans for health components of development projects in 1975. The Bank's experience from 1975 through 1978—involvement in 70 health components of development projects in 44 countries—prompted the Bank in 1980 to begin lending directly for health projects.

The economic reasons for supporting health projects are set forth in the second edition of the World Bank's *Health: Sector Policy Paper* (412). The economic effects of ill health, on which the Bank bases its policy, are summarized below.

1. **Reducing Availability of Labor.** The effective labor force is reduced by premature death and by illness. The death of workers may not have a national impact on productivity in developing countries, where unemployment is high and there is a steady supply of replacements. Premature death may, however, impose some costs on society when dependents are left.

Morbidity of workers, resulting in absenteeism, has a more direct effect on overall productivity. That effect has been measured in a few studies, though it is generally difficult to prove. Antimalaria programs in the Philippines and parts of Africa, and yaws control in Haiti have substantially reduced absenteeism (412).

2. **Impairing Productivity of Labor.** The ability of a sick worker is impaired not only in terms of physical capacity, but also in terms of the capacity to concentrate and think. The World Bank studied construction and rubber plantation workers in Indonesia, 85 percent of whom had hookworm infestations. After the workers were given iron supplements to correct the anemia caused by the worms, productivity increased 19 percent, for a cost of only 13 cents per worker over 12 months (17).

At a more elemental level, the ability to learn, at all levels of education and training, is impaired by ill health. Absence from school compounds the negative effect of sickness.

3. **Wasting Current Resources.** Resources are used up in often ineffectual treatments of diseases. Even when effective, many treatments are costly and pull disproportionate amounts from the overall health budget. Many illnesses also cause an increase in calorie use by the body, using up scarce food resources, and in turn contributing to malnutrition.

4. **Impeding Development of Resources.** Some areas of the world, particularly parts of Africa, are effectively closed to development because of disease threats. Some areas have been settled only to be abandoned because of the lack of effective control measures for diseases. African sleeping sickness (African trypanosomiasis) and river blindness (onchocerciasis) render parts of Africa uninhabitable by humans and/or domestic animals. The World Bank and the World Health Organization (WHO) have undertaken vector control programs to open some areas. Making areas safe for inhabitants also can increase the attractiveness of a country for tourists, a definite economic advantage.

Photo credit: Dr. Stanley O. Foster, Centers for Disease Control

The eradication of smallpox, through a WHO-led worldwide vaccination campaign, has saved an estimated $1 to $2 billion each year since 1977.

To the general economic benefits of improved health can be added some direct economic benefits to the United States, though explicit examples are few. The classic is the cost-savings accruing to the United States (and the rest of the world) as a result of the smallpox eradication program. The United States spent about $32 million on smallpox eradication over 10 years; that amount is recouped *every 3 months* in savings (161). In 1976, D. A. Henderson, who was head of the program, wrote (151):

> What of the cost of the 10-year campaign? Approximately $83 million has been spent in international assistance for the smallpox eradication program since 1967. The endemic countries themselves have spent roughly twice that amount, but few of them have spent much more than they were already spending on smallpox control. The total amount of money spent in international assistance is little more than half what was computed in 1968 to be the yearly expenditure for smallpox control in the U.S. alone; worldwide expenditures for smallpox vaccination and quarantine measures have been estimated as being in the range of from $1 billion to $2 billion a year. With the eradication of the disease, smallpox vaccination will no longer be required, nor will international certificates of smallpox vaccination. Apart from the alleviation of human suffering, the savings have already repaid the small investment many times over.

In addition to directly saving money, improved health can lead to a generally higher quality of life and, ultimately, to improvements in the economic development of a country.

Scientific Objectives

The tropical diseases of particular scientific interest today are the parasitic diseases, mainly those caused by protozoa (e.g., malaria, trypanosomiasis, leishmaniasis) and helminths (e.g., filariasis, schistosomiasis). While U.S. research on these diseases is still at a very low level in comparison with research on diseases of domestic importance, scientific interest has been heightened by advances in immunology and biotechnology. The parasites that cause tropical diseases are more complex immunologically than the organisms that cause infectious diseases common in the United States, and these parasites have captured the attention of both basic and applied scientists. Their research activities are highlighted in this report, particularly in chapters 6, 7, and 8.

In a more general sense, scientists want their work to have an effect, and such desires do not stop at political boundaries. Advancing the control of diseases in developing countries is a great scientific challenge, and success in meeting it would be richly rewarding in terms of bettering the human condition.

U.S. SUPPORT OF INTERNATIONAL HEALTH ACTIVITIES

For the past four decades, U.S. Presidents and Members of Congress have shown sporadic enthusiasm for international health activities. Nearly every President since Truman has spoken out for a U.S. role in international science and international health. From President Truman's "Point Four Program" through President Carter's assertion that "Health is a basic human right," improvements in health have been seen as part of the solution to social, political, and economic problems. Congress has acted similarly, but the pronouncements and legislation have not been matched by funding for new programs.

One observer summarized the history of the interactions as follows (75):

> . . . the process over a period of time has become cyclical. The Legislative and Executive branches have not been able to agree on a course of action because the enthusiasm of one branch of Government has been asynchronous with that of the other. Furthermore, the Office of Management and Budget has deterred any Department- or Agency-generated initiatives which would entail increased expenditures abroad.

The International Health Research Act of 1960

In 1958, Senator Lister Hill introduced a bill to institute broad international programs in health research. Congressman John Fogarty introduced an identical bill in the U.S. House of Representatives. The specific measures called for: 1) a National Advisory Council for International Medical Research; 2) a National Institute for International Medical Research as part of the National Institutes of Health (NIH); and 3) a $50 million annual budget authority for international medical research.

Senator Hill stressed the need not only for research, but for information exchange and for training of research personnel. He contrasted his $50 million budget authority for international health research with the $400 million then being spent on medical research in the United States and the $40 billion for defense that had just been authorized.

The bill passed the Senate, but the House made changes consistent with the wishes of the Eisenhower Administration and removed many of the specific provisions. The objections to the Senate version, according to Corning, were as follows (75):

> . . . too much money; a new institute at the NIH for its administration was unnecessary; the international program authorized by the bill was considered to be a foreign policy matter; and the proposed program should be linked with the Department of State and the International Cooperation Administration and executed under the immediate supervision of the President.

The House version passed and became the International Health Research Act of 1960. An important distinction was made in the revision to separate international health activities that benefit the United States from those designed to advance the status of international health as a whole. Authority for activities benefiting the United States was delegated to the Secretary of Health, Education, and Welfare (now Health and Human Services). Authority to engage in activities for the purpose of advancing international health rests with the President and extends only to research and training for research, not operational health programs.

Some research conducted by NIH under the broader authority of the Public Health Service Act (the legislation that provides overall direction to Public Health Service activities) is of great benefit to other countries. Current work in the broad field of immunology is a case in point. The International Health Research Act, however, still provides the basis for most U.S. Department of Health and Human Services activities in international health and places limits on the initiatives that may be taken by NIH and the Centers for Disease Control (CDC).

The most recent forceful assertion of the importance of international health came under President Carter. In 1978, the White House Office of Science and Technology Policy produced the report *New Directions in International Health Cooperation* (27). Federal agencies responded to that report by proposing several initiatives for domestic and multilateral programs in international health. The proposals included research in tropical medicine, establishing a global epidemic intelligence service, supporting the U.N. Development Program/World Bank/WHO Special Program for Research and Training in Tropical Diseases (TDR), developing a worldwide immunization program, establishing a health unit to respond to disasters around the world, encouraging the pharmaceutical industry to be more responsive to the needs of the developing world, and establishing nutrition surveillance and research programs. In spite of the enthusiasm, no new funds were allocated for these purposes, and progress was made only to the extent that existing budgets could support it, which for the most part was relatively little.

According to one observer, "the United States has been sensitive to international health needs, has had continuing concern, but has not always addressed the issue of availability of resources and responded with active programs" (75). That observation continues to be accurate (see ch. 3).

Support of Multilateral Efforts

The United States has an admirable record in supporting multilateral international health efforts. Since the Pan American Sanitary Bureau was established in 1902, the United States has provided major financial support (about 65 percent of the regular annual budget) and technical assistance to it and to the later Pan American Health Organization. The United States energetically promoted and participated in the formation and funding of WHO beginning in the mid-1940s. The United States is currently a major supporter of TDR (see ch. 3).

U.S. REPORTS ON TROPICAL DISEASE RESEARCH AND TRAINING

The most comprehensive look at tropical disease research was a 1962 report by the National Academy of Sciences/National Research Council (NAS/NRC). A current NAS study is addressing the U.S. capacity to address tropical disease problems. These reports are discussed further below.

1962 NAS/NRC Report on Tropical Health

The most comprehensive look at tropical disease research, training, and practice, and the resources put toward those ends was taken more than 20 years ago in an NAS/NRC report entitled *Tropical Health: A Report on a Study of Needs and Resources* (252).

In the mid-1950s, the American Society for Tropical Medicine and Hygiene (ASTMH) recognized that interest in tropical medicine had begun to decline. The boost given by World War II, interest generated from practical necessity, had ceased to have effect. Money for research was drying up, and medical schools were cutting back the teaching of tropical medicine. At the same time, the United States was developing its foreign aid programs to include health. Without continuing support for research and training for the health problems of recipient countries, mainly those in which tropical diseases are endemic, the professionals necessary to implement programs would not be available.

Dr. Albert Sabin, who chaired an ASTMH committee to consider these problems, was largely responsible for enlisting NRC to carry out its study. Funding was provided by NIH, the U.S. Army Research and Development Command, and the Rockefeller Foundation.

The 1962 NRC report is encyclopaedic in scope. Geographically, the five major regions covered are: 1) the Caribbean and Central and South America, 2) Africa, 3) Southwest Asia, 4) South Central and Southeast Asia, and 5) Oceania. In 20 chapters, the report discusses the major human and animal diseases, including what is known of their incidence, prevalence, and mortality rates by region, and the status of prevention and treatment; resources for health and medical care; impacts of tropical health and disease on the United States; U.S.-based and foreign research programs on tropical diseases; research grant and fellowship programs in tropical health; the teaching of tropical medicine and hygiene and facilities for training; and career opportunities and future manpower needs.

Research recommendations for each disease are presented as an "assembly of informed opinion," gathered by polling experts worldwide. Very specific suggestions are included, covering all aspects of laboratory, field, and clinical research.

The committee responsible for the report also drew up several broad resolutions addressed to various public and private entities in the United States, specifically (252):

1. Pave the way to increased U.S. participation in international health activities, if necessary through legislation. Solutions to disease problems of the tropics require systematic research and development programs. The United States, by virtue of its position of world leadership has an obligation to contribute to the fullest extent to research and training activities. Participation should not be tied to direct benefits to the United States.
2. Explore creation of an advisory group within NAS/NRC to organize support for a "National Program for Research in Tropical Health," with involvement of the relevant government agencies and private organizations.
3. Seek authority to make direct grants to foreign institutions for support of research, development, or training in tropical health.
4. Strengthen undergraduate and postgraduate medical training in tropical diseases. This could include opportunities for training in the tropics.
5. Seek to improve statistics relating to tropical diseases, by working through WHO and by offering WHO increased U.S. support to accomplish this aim.
6. Encourage studies to document economic loss due to disease and gains accruing from disease control.
7. Encourage and provide additional support for international veterinary health organizations.

The 1962 NAS/NRC study was, and still is, of inestimable value to people in the field, giving a clear picture of the state of knowledge at the time. That study is now the benchmark against which progress can be measured. But it does not appear to have had a significant impact on U.S. Government policies or practices.

Legislation to foster U.S. involvement in tropical health has not been passed. The advisory group suggested in resolution 2 was not formed. Funding for research, and particularly for developing country institutions, is still passively allowed in most cases, rather than actively encouraged. There is widespread agreement that the U.S. capacity to train tropical health professionals has eroded, if anything, in recent years.

On the positive side, statistics regarding health conditions in the tropics have gradually become more reliable. The major improvements have been in birth and death reporting, and not so much in the inherently more difficult task of recording nonfatal cases of disease. The number of studies of the type recommended in resolution 6 that have been carried out could probably be counted on one hand.

Current NAS Study of U.S. Capacity To Address Tropical Disease Problems

Leaders in tropical medicine and tropical public health have expressed alarm at the current erosion of U.S. support for training in those fields. The capacity of the United States to train people in parasitology, vector control, tropical medicine, and related fields in tropical public health is reaching a low ebb. This crisis is the subject of a current study by NAS.

The NAS study, *U.S. Capacity To Address Tropical Disease Problems*, is being carried out by NRC's Board on Science and Technology for International Development (BOSTID), in cooperation with the Institute of Medicine (IOM). IOM was approached about the need for this study by an ad hoc committee of ASTMH.

The NAS study has several objectives:

- to examine current capabilities of U.S. centers to conduct research and postgraduate training in tropical medicine and tropical public health;
- to estimate future manpower needs in applied research and training; and

- to assess the adequacy of mechanisms for providing research and training support in the field.

Funding for the study is being provided by the U.S. Army Research and Development Command, the National Institute for Allergy and Infectious Diseases, CDC, and AID. The Rockefeller Foundation will cosponsor and support an international workshop in connection with the study. A report from the study is expected in late 1985. This report should provide information on which to make decisions about the types of funding mechanisms and programs that could be emphasized or mounted in future funding decisions. There will still be a need to examine the opportunities for application of available technologies and the constraints to their application.

TROPICAL DISEASE RESEARCH IN THE CONTEXT OF U.S. INTERNATIONAL HEALTH ACTIVITIES

Efforts to improve international health consist of a great deal more than research, and in fact, research is a small component in terms of money spent and personnel deployed. Most U.S. international health assistance is directed toward operational activities—particularly in providing primary health care, environmental services, and nutrition and population programs. These activities are closely tied to U.S. foreign policy. Guidelines for operational activities are established by the Department of State, and the activities themselves are largely carried out by AID. Programs and resources are provided not solely on the basis of need, but in accordance with U.S. foreign policy goals.

Much of the published analysis of the U.S. international health effort relates more closely to operational activities than to research. Tropical disease research shares some but not all of the problems of the larger field of international health and is also subject to other influences.

One concern of many observers of international health is the lack of a central Federal policy stating U.S. goals for international health activities. There is a statement of policy for research, which is stated in the International Health Act of 1960—but that policy is fairly weak (see discussion above). The lack of an overall U.S. policy subordinates international health aims to the general mandates and strictures of each agency as it carries out its activities.

Furthermore, lack of a clear-cut international health policy results in tropical disease research remaining low in status in relation to domestic biomedical research. Operational programs can use the leverage of foreign policy benefit to support their activities, but research programs, which may have less direct benefits, may be more difficult to justify.

A strong central policy supporting an aggressive U.S. role in international health could have a salutary effect on all aspects of the field, including tropical disease research. However, the effect of such a policy would probably be greater in expanding operational programs than in expanding research. The reason is that operational programs have a more immediate, tangible, foreign policy impact than do research programs. A program in a particular country in need of health assistance is a more dramatic display of U.S. interest than is a researcher at NIH searching for long-term solutions to disease problems. For this reason, if a policy statement is considered by the U.S. Government, the intent of the policy concerning research, as distinct from operational aims, should probably be spelled out.

3.

Funding of Tropical Disease Research

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3.

Funding of Tropical Disease Research

INTRODUCTION

This chapter presents an analysis of funding of biomedical research in the tropical diseases of interest in this assessment—namely, malaria, schistosomiasis, trypanosomiasis, leishmaniasis, filariasis, leprosy, tuberculosis, diarrheal diseases, acute respiratory infections (ARIs), and diseases caused by arboviruses.

The chapter describes the major funding sources and levels of funding for tropical disease research. It also discusses the types of research (by disease and by research goals) that the funding sources support. The concluding section of the chapter presents information about the types of organizations that actually conduct the research.

The analysis in this chapter represents the first attempt in over 20 years to review all major funding sources for tropical diseases. The information in this analysis was obtained from various sources that do not report according to a common set of definitions or in a common format. Every attempt has been made to take account of variations and to identify assumptions and accommodations that have been made. Even so, the data presented in this chapter should be regarded as indicative rather than definitive. Additional information about the limitations of the data is presented in appendix B.

FUNDING SOURCES

The sources of tropical disease research funding considered in this analysis are as follows:

- the Special Program for Research and Training in Tropical Diseases (TDR) and the World Health Organization (WHO);
- U.S. Government agencies, specifically, the Department of Health and Human Services (DHHS), the Agency for International Development (AID), and the Department of Defense (DOD); and
- U.S. private foundations.

Private pharmaceutical companies are discussed separately.

Each source of tropical disease research funding considered in this analysis is profiled below. Data made available by the funding sources themselves are analyzed to show the level of funding, historical trends, and the allocation of funding across the tropical diseases of interest.

Special Program for Research and Training in Tropical Diseases (TDR)

TDR was planned and initiated by WHO, with the assistance and cosponsorship of the U.N. Development Program (UNDP) and the World Bank. The program was initiated in 1974, and between that date and the end of 1982, nearly $120 million had been contributed to the program by 25 governments, other organizations, the World Bank, UNDP, and WHO. The U.S. contribution alone was about $15 million, and by fiscal year 1983, the United States had contributed a total of about $20 million over a 7-year period (351,353).

TDR has two interdependent objectives (353):

- to develop new and improved tools to control six tropical diseases: malaria, schistosomiasis, trypanosomiasis, leishmaniasis, filariasis, and leprosy; and

- to strengthen the biomedical research capabilities of developing countries.

TDR is organized into four program areas: 1) Administrative and Technical Bodies; 2) Research and Development; 3) Research Capability Strengthening; and 4) Program Management.

TDR's Research and Development program area has 10 components: one component for each of the six specific tropical diseases and four additional components that cut across disease lines. The four transdisease components of TDR's Research and Development program area are biomedical sciences, vector control and biology, epidemiology, and social and economic research. Scientific working groups make recommendations about program direction and the allocation of research funding.

Table 3-1 presents information about the overall growth of the disease-specific components of TDR's Research and Development program area from 1977 through 1981. (These components of the program area comprised about 85 percent of the program area's total expenditures from 1977 to 1981.) The data in table 3-1 show that the disease-specific components of TDR's Research and Development program area grew from 128 projects amounting to $2,791,000 in 1977 to 374 projects amounting to $10,377,000 during 1981.

From 1977 through 1981, TDR allocated a total of some $43,767,000 to investigations into the six diseases (351). The percentage of this research funding allocated to specific diseases from 1977 to 1981 was as follows: malaria, 30 percent; schistosomiasis, 14 percent; trypanosomiasis, 22 percent; leishmaniasis, 6 percent; filariasis, 16 percent; and leprosy, 13 percent. The percentage of funding for research on specific diseases remained fairly constant each year, with the exceptions of a decline for schistosomiasis from 16 percent in 1979 to 10 percent in 1981 and an increase for leprosy from 11 percent in 1979 to 16 percent in 1981.

World Health Organization (WHO)

Research on two groups of diseases discussed in this report—ARIs and diarrheal diseases—is funded through separate WHO programs.

In the biennium 1980-81, WHO allocated a total of $2,389,000 for biomedical reseach pertaining to tropical diseases of interest in this assessment (see table 3-2). This research falls in the broad category of communicable disease prevention and control. The data in table 3-2 were collected by a combination of budget review and personal interviews and should be regarded as approximations (108). Sixty-six percent of the funding for such research came from regular budgetary sources.

The United States contributes 25 percent of the regular WHO budget, just under $400,000 for the 1980-81 period. The United States (as do other countries and organizations) makes additional allocations on a program-by-program basis (extrabudgetary contributions), bringing the total annual contribution of the United States to between $250,000 and $500,000 for 1980-81. An example of other extrabudgetary WHO funds in 1980-81 was $600,000 for research into leprosy, much of which was a gift from the Japan Shipbuilders Association.

WHO Program in Acute Respiratory Infections

In 1980-81, WHO spent a total of $381,000 for research in ARIs and tuberculosis, 45 percent of which was from regular budgetary sources (see table 3-2). In 1983, ARI was identified as a distinct WHO program area. Since then, while ARIs have been receiving greater attention within the WHO regular budget, support from extrabudgetary sources has been relatively modest. The Pan American Health Organization (PAHO) reports that in 1983 its program for ARIs received only $31,000 in direct support funds from WHO, and a further $45,000 for tuberculosis (207).

WHO Program in Diarrheal Diseases

WHO's Program for Control of Diarrheal Diseases (CDD) was initiated in 1978. Its objectives are to reduce the mortality and morbidity caused by acute diarrheal diseases and to promote the self-reliance of individual countries in the delivery of health and social services for the control of diarrheal diseases (427).

Table 3-1.—TDR's Research and Development Program Area: Distribution of Projects[a] [b] and Budget Amounts for Specific Tropical Diseases, 1977-81

	1977			1978			1979			1980			1981			Total 1977-81	
Disease	Number of projects	Budget amount (000s)	Percent of 1977 budget amount	Number of projects	Budget amount (000s)	Percent of 1978 budget amount	Number of projects	Budget amount (000s)	Percent of 1979 budget amount	Number of projects	Budget amount (000s)	Percent of 1980 budget amount	Number of projects	Budget amount (000s)	Percent of 1981 budget amount	Budget amount (000s)	Percent of 1977-81 budget amount
Malaria	29	$ 886	32%	71	$2 644	30%	112	$ 3 084	29%	118	$ 3 161	29%	105	$ 3 203	31%	$12 977	30%
Schistosomiasis	18	456	16	50	1 263	14	62	1 745	16	53	1 578	14	40	1 044	10	6 085	14
Trypanosomiasis	1	10	<1	58	2 266	25	85	2 505	23	85	2 337	21	80	2 335	23	9 454	22
Leishmaniasis	0	0	0	29	598	7	45	612	6	51	694	6	39	614	6	2 518	6
Filariasis	35	909	33	49	1 274	14	55	1 680	16	52	1 684	15	43	1 548	15	7 094	16
Leprosy	45	530	19	66	882	10	67	1 153	11	66	1 440	13	67	1 633	16	5 639	13
Total by year	128	$2 791	100%	323	$8 927	100%	426	$10 779	100%	425	$10 894	100%	374	$10 377	100%	$43 767	100%

[a] In addition, a total of 229 other projects were funded under Director's Initiative Fund, Biomedical Sciences, Vector Biology and Control, Epidemiology and Social and Economic Research. Funding for these projects amounted to approximately $7 762 000, or 15 percent of the total budget for research projects during this period.

[b] A project approved for a duration longer than 1 year is counted in each year for which it is funded.

SOURCE: Derived from U.N. Development Program/World Bank/World Health Organization, Special Program for Research and Training in Tropical Diseases, Facts and Figures, No. 6, Summary Tables (Geneva: WHO, January 1982), pp. 9-10.

Table 3-2.—WHO Funding for Selected Biomedical Research Pertaining to Tropical Diseases, 1980-81 Biennium

Type of research	Amount of funding (000s) Regular budget	Extra-budgetary	Total budget
Malaria	$ 222	$ 11	$ 233
Other parasitic diseases	335	0	335
Bacterial diseases, plague, meningitis and mycotic diseases	105	0	105
Leprosy	66	600	666
Tuberculosis and acute respiratory infections (ARIs)	170	211	381
Viral diseases	250	0	250
Vector biology and control	419	0	419
Total	$1,567	$822	$2,389

SOURCE: Data presented in V. Elliott and P. Contacos, "A Profile of Selected Biomedical Research Efforts Into Diseases of Major Public Health Importance to People of Developing Countries," typescript, prepared for the U.S. Agency for International Development, Washington, DC, November 1982.

Support for CDD is predominantly from extrabudgetary WHO sources. In the biennium 1980-81, CDD resources totaled $6,879,018, of which $5,004,840 (73 percent) came from extrabudgetary sources. In 1982, total resources were $7,857,429 of which $6,932,352 (88 percent) were extrabudgetary. The contribution of the United States was $157,400 in 1980-81 and $78,800 in 1982.

CDD obligated a total of $5,654,423 during 1980-81 and an estimated $14,373,600 during 1982-83. Of these obligations, 33 percent in 1980-81 and 39 percent in 1982-83 were set aside for research. The research component of CDD includes both biomedical and operational activities. In 1980-81, $1,060,095 (56 percent) of CDD's research budget was allocated to biomedical research; estimates for 1982-83 are $3,814,700 (67 percent) (427). These figures include funds for planning, coordination, and support of collaborating centers as well as direct support of research projects.

Table 3-3 shows that between 1980 and 1983, CDD provided $3,035,000 in funds for a total of 149 biomedical research projects. Bacterial enteric infections were the focus of 63 of these research projects and accounted for $1,150,000 (38 percent) of research project funds over the period. Drug development and the management of acute diarrheas accounted for 25 percent of these funds and viral diarrheas for somewhat less (23 percent) (425).

Table 3-3.—WHO Program for the Control of Diarrheal Diseases (CDD): Funding for Biomedical Research Projects, 1980-83

Type of research	Number of projects 1980-83	Budget amount (000s) 1980-81	1982	1983	Total 1980-83 budget amount (000s)	Percent of total 1980-83 budget amount
Bacterial enteric infections	63	$222	$312	$ 616	$1,150	38%
Parasitic diarrheas	8	0	10	111	121	4
Viral diarrheas	36	61	256	390	707	23
Drug development and acute diarrhea management	31	109	159	497	765	25
Other	11	25	119	148	292	10
Total by year	149	$417	$856	$1,762	$3,035	100%

SOURCE: Derived from World Health Organization, *Program for Control of Diarrheal Diseases*, DCC/TAG/84.2A (Geneva: WHO, February 1984).

U.S. Department of Health and Human Services (DHHS)

Three organizational units within DHHS fund research in the tropical diseases of interest in this assessment:

- the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH);
- the Centers for Disease Control (CDC); and
- the Fogarty International Center for Advanced Study in the Health Sciences of NIH.

The activities of these organizations are discussed below.

National Institute of Allergy and Infectious Diseases

NIAID has primary responsibility for tropical medicine research at NIH. In fiscal year 1981, the total NIAID budget accounted for $232 million (or 6 percent) of the total NIH obligations of $3,572 million (383). That year, as shown in table 3-4, NIAID awarded about $26,865,000 in extramural and intramural grants for research activities in tropical diseases, general parasitology, and general tropical medicine (379). These grants represented about 12 percent of NIAID funds and less than 1 percent of all NIH funds in fiscal year 1981.

Table 3-4 shows that overall NIAID funding for tropical disease research increased from a level of almost $27 million in fiscal year 1981 to a little more than $33 million in fiscal year 1983 (379, 380,381). This NIAID-funded research can be classified under three general headings: 1) tropical diseases (malaria, schistosomiasis, trypanosomiasis, leishmaniasis, filariasis, and leprosy); 2) general parasitology (cestodes, nematodes, protozoa, and trematodes); and 3) general tropical medicine (rickettsia, bacteriology, mycology, virology, and vector pathogens). As shown in table 3-4, the percentage of funds allocated to research under the

Table 3-4.—NIAID Funding for Tropical Disease Research, Fiscal Years 1981-83

	Amount of funding (000s)		Combined extramural and intramural funding	
Fiscal year and program area	Extramural	Intramural	Amount (000s)	Percent
Fiscal year 1981:				
Tropical diseases	$ 9,101	$4,187	$13,288	49%
General parasitology	3,551	1,163	4,714	18
General tropical medicine	7,660	1,203	8,863	33
Total	$20,312	$6,553	$26,865[a]	100%
Percent of combined funding amount	76%	24%	100%	
Fiscal year 1982:				
Tropical diseases	$10,042	$5,627	$15,670	51%
General parasitology	3,502	1,854	5,356	17
General tropical medicine	7,659	1,991	9,650	31
Total	$21,203	$9,472	$30,676[b]	100%
Percent of combined funding amount	69%	31%	100%	
Fiscal year 1983:				
Tropical diseases	$16,211	$5,182	$21,393	65%
General parasitology	2,908	1,245	4,153	13
General tropical medicine	6,182	1,430	7,612	23
Total	$25,301	$7,857	$33,158[c]	100%
Percent of combined funding amount	76%	24%	100%	

[a] In fiscal year 1981, $40,000 of this amount was for research into acute respiratory infections (ARIs) and $106,000 for research into diarrheal and enteric infections.

[b] In fiscal year 1982, $930,000 of this amount was for research into ARIs and $2,464,000 for research into diarrheal and enteric infections.

[c] In fiscal year 1983, $3,019,863 of this amount was for research into arboviruses, $1,080,000 for research into ARIs, and $2,850,000 for research into diarrheal and enteric infections.

SOURCE: U.S. Department of Health and Human Services, National Institutes of Health, "International Cooperation by the National Institute of Allergy and Infectious Diseases, FY 1981, FY 1982, FY 1983," mimeo, Bethesda, MD, no date.

tropical diseases heading rose from 49 percent in fiscal year 1981 to 65 percent in fiscal year 1983, while the percentage allocated to general tropical medicine declined from 33 percent in fiscal year 1983 to 23 percent in fiscal year 1983. About three-quarters of NIAID funding for tropical disease research was awarded through extramural grants and contracts in fiscal years 1981 and 1983 and a slightly smaller proportion in fiscal year 1982. There were no major differences in the proportion of extramural awards between the three categories of research.

Table 3-5 shows the allocation of NIAID funds for research under the heading of tropical diseases—i.e., on the six TDR diseases—for fiscal years 1981, 1982, and 1983. Funds for research into each of these diseases increased over the 3-year period, with the exception of funds for leprosy research, which were substantially reduced in fiscal year 1982, but increased again in fiscal year 1983. Over the 3-year period, trypanosomiasis research accounted for 26 percent of NIAID funding for research on the six TDR diseases; schistosomiasis research, 22 percent; malaria research, 20 percent; leishmaniasis research, 18 percent; filariasis research, 9 percent; and leprosy research, 5 percent. The same relative level of NIAID research funding among the six diseases is seen for each year for which data are presented, with the exception of fiscal year 1983, when funding for leishmaniasis research slightly exceeded that for research in malaria.

Table 3-6 shows NIAID funding for research on diarrheal and enteric diseases and ARIs, through grants awarded under the headings of general parasitology and general tropical medicine, for fiscal years 1981, 1982, and 1983. Most NIAID funding for research in diarrheal and enteric diseases and ARIs is considered domestic research for reporting purposes, but the share allocated to tropical medicine has increased. In fiscal year 1981, only 1 percent of NIAID funds for research in diarrheal and enteric diseases and ARIs came under the heading of tropical medicine; in 1982, 16 percent; and in 1983, 15 percent (263).

The data presented in table 3-6 show that NIAID funded research on ARIs at more than twice the funding level for research on diarrheal and enteric infections during the 3-year period 1981-83. The distribution of funding between extramural and intramural research for these two disease classes varied during the 3-year period, ending with a slight shift toward extramural research in fiscal year 1983.

Centers for Disease Control

CDC conducts research in all of the categories of tropical diseases of interest in this assessment. Table 3-7 shows the levels of CDC funding of such research during fiscal years 1981, 1982, and 1983. Overall, CDC's tropical disease research funding grew from $3,877,000 in fiscal year 1981 to $4,929,000 in fiscal year 1983 (288). This growth is reflected in each of the categories of disease shown in the table. Overall, about 44 percent of CDC's tropical disease research funding from 1981 to 1983 was allocated to research on the six TDR diseases; 22 percent was allocated to research on diarrheal diseases; 29 percent to research on ARIs; and 4 percent to research on arboviral infections.

Table 3-5.—NIAID Funding Specifically for Research on the Six TDR Diseases, Fiscal Years 1981-83

	Fiscal year 1981		Fiscal year 1982		Fiscal year 1983		Fiscal years 1981-83	
Disease	Amount[a] (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent
Malaria	$ 2,426	18%	$ 3,567	23%	$ 3,950	18%	$ 9,943	20%
Schistosomiasis	2,984	22	3,550	23	4,598	21	11,132	22
Trypanosomiasis	3,783	28	3,889	25	5,308	25	12,980	26
Leishmaniasis	1,927	15	2,883	18	4,107	19	8,917	18
Filariasis	1,189	9	1,452	9	2,051	10	4,692	9
Leprosy	979	7	330	2	1,379	6	2,688	5
Total by year	$13,288	100%	$15,670	100%	$21,393	100%	$50,351	100%

SOURCE: U.S. Department of Health and Human Services, National Institutes of Health, "International Cooperation by the National Institute of Allergy and Infectious Diseases, FY 1981, FY 1982, FY 1983," mimeo, Bethesda, MD, no date.

Table 3-6.—NIAID Funding for Research on Diarrheal and Enteric Infections and Acute Respiratory Infections (ARIs), Fiscal Years 1981-83

	Extramural funding amount (000s)	Intramural funding amount (000s)	Combined funding amount (000s)
Fiscal year 1981:			
Diarrheal and enteric infections	$ 4,459	$2,497	$ 6,956
ARIs	11,819	4,442	16,261
Total	$16,278	$6,939	$23,217
Percent of combined funding amount	70%	30%	100%
Fiscal year 1982:			
Diarrheal and enteric infections	$ 4,195	$2,984	$ 7,179
ARIs	10,194	4,293	14,487
Total	$14,389	$7,277	$21,666
Percent of combined funding amount	66%	34%	100%
Fiscal year 1983:			
Diarrheal and enteric infections	$ 5,452	$2,731	$ 8,183
ARIs	14,689	3,944	18,633
Total	$20,141	$6,675	$26,816
Percent of combined funding amount	75%	25%	100%

SOURCE: J. E. Nutter, Chief, Office of Program Planning and Evaluation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, personal communication, April 1984.

Table 3-7.—CDC Funding for Tropical Disease Research, Fiscal Years 1981-83

	Fiscal year 1981		Fiscal year 1982		Fiscal year 1983		Fiscal years 1981-83	
	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent
TDR diseases[a]	$1,722	44%	$1,927	45%	$2,160	44%	$ 5,809	44%
Diarrheal diseases	859	22	976	23	1,113	23	2,948	22
ARIs	1,130	29	1,261	29	1,397	28	3,788	29
Arboviral infections	166	4	166	4	259	5	591	4
Total by year	$3,877	100%	$4,330	100%	$4,929	100%	$13,136	100%

[a] Malaria, schistosomiasis, trypanosomiasis, filariasis, leishmaniasis, and leprosy.

SOURCE: J. G. Randolf, Budget Analyst, Financial Management Office, Centers for Disease Control, Atlanta, GA, personal communication, April 1984.

Table 3-8 shows the amount and distribution of CDC funds among the six TDR diseases for fiscal years 1981-83. Over that 3-year period, research in malaria received more than 60 percent of this funding. Schistosomiasis research funding increased from 15 percent of the funds in 1981 to 21 percent in 1983, while filariasis research funding declined from 6 percent of the funds in 1981 to 4 percent in 1983.

Fogarty International Center for Advanced Study in the Health Sciences

FIC is the focal point within NIH for scientific exchange and collaboration at the international level. The FIC budget was $5.1 million in fiscal year 1979, $4.5 million in fiscal year 1980, and $4.3 million in fiscal year 1981 (382). Most of the activities of FIC fall outside the biomedical research concerns that are the subject of this analysis.

A major exception, however, is FIC's responsibility for transmitting the U.S. Government's care support to the Gorgas Memorial Institute of Tropical and Preventive Medicine. The operating unit of the institute is the Gorgas Memorial Laboratory, located in Panama. Neither FIC nor any other U.S. agency has programmatic control over the activities at the laboratory. Gorgas received $1.7 million in core support through FIC in fiscal year 1980, $1.8 million in fiscal year 1981, $1.692 million in fiscal year 1982, and $1.8 million in fiscal year 1983 (360). These amounts con-

Table 3-8.—CDC Funding for Research on the Six TDR Diseases, Fiscal Years 1981-83

	Fiscal year 1981		Fiscal year 1982		Fiscal year 1983		Fiscal years 1981-83	
Disease	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent
Malaria	$1,127	65%	$1,208	63%	$1,290	60%	$3,625	62%
Schistosomiasis	250	15	352	18	461	21	1,063	18
Trypanosomiasis	40	2	48	2	58	3	146	3
Leishmaniasis	66	4	66	3	98	5	230	4
Filariasis	101	6	101	5	86	4	288	5
Leprosy	138	8	152	8	167	8	457	8
Total by year	$1,722	100%	$1,927	100%	$2,160	100%	$5,809	100%

SOURCE: J. G. Randolf, Budget Analyst, Financial Management Office, Centers for Disease Control, Atlanta, GA, personal communication, April 1984.

stitute between 64 and 74 percent of all support received by the institute. Other agencies of the U.S. Government also provide funds to Gorgas Memorial Institute through grants and contracts. Further information about Gorgas is presented in OTA's technical memorandum entitled *Quality and Relevance of Research and Related Activities at the Gorgas Memorial Laboratory* (360), which was published in August 1983 as part of this assessment.

U.S. Agency for International Development (AID)

AID's Bureau for Science and Technology supports a variety of biomedical research activities pertaining to tropical diseases. Table 3-9 presents information for fiscal years 1979 through 1984 about projects that are classified by AID as "Disease Control—Research" (152). This category includes AID's support for TDR and for the International Center for Diarrheal Disease Research, Bangladesh. In addition to providing this institutional support, AID funds research through grants and contracts. The research activities funded by AID in the past have been primarily concerned with malaria immunology and vaccine development, but AID is planning to expand its tropical disease research agenda (110).

Since 1981, the Office of the Science Advisor of AID has also been supporting biomedical research through AID's Program in Science and Technology Cooperation (PSTC) and through the complementary Research Grants Program of the National Academy of Sciences' Board on Science and Technology for International Development (BOSTID). These programs administer grant funds which are intended to stimulate investiga-

Table 3-9.—AID's Bureau for Science and Technology: Funding for Disease Control Research Projects, Fiscal Years 1979-84

	Amount of funding (000s)					
Project title	Fiscal year 1979	Fiscal year 1980	Fiscal year 1981	Fiscal year 1982	Fiscal year 1983	Fiscal year 1984
Malaria immunity and vaccine research	$1,773	$2,679	$ 4,203	$3,000	$ 5,787	$3,900
Comprehensive methods of vector control	0	0	0	40	200	500
Alternatives to DDT	0	198	281	300	0	0
Antischistosomal drug testing	0	0	100	0	0	0
Tropical diseases research[a]	1,500	4,000	4,000	4,000	3,000	2,000
International Center for Diarrheal Disease Research (Bangladesh)	2,060[b]	1,900	1,900	1,900	2,217	0
Diarrheal disease research	0	0	0	0	0	1,900
Total	$5,333	$8,777	$10,484	$9,240	$11,204	$8,300

[a] This project provides support to the U.N. Development Program/World Bank/WHO TDR program.

[b] In fiscal year 1980, AID support for the International Center for Diarrheal Disease Research (Bangladesh) came from the Asia Bureau ($1,400,000) and from the Bureau for Science and Technology ($660,000). In other fiscal years, the support was from the Bureau for Science and Technology.

SOURCE: F. R. Herder, Deputy Director for Health and Population, U.S. Agency for International Development, Washington, DC, personal communication, March 1984.

tion of problems that confront developing countries. The Research Grants Program administered by BOSTID provides funds only to developing country institutions. PSTC gives priority to proposals received from developing countries, but also funds activities in the United States.

PSTC has concentrated on five areas of research. One of these relates to tropical diseases—namely, biotechnology/immunology and biological control of human schistosomes and associated snail vectors (73).

The BOSTID program concentrates on six areas of research. Three of these areas—field studies of the mosquito vector, rapid epidemiologic assessment, and the diagnosis and epidemiology of ARIs in children—fall within the broad rubric of biomedical research in tropical diseases.

Table 3-10 shows AID funds committed to PSTC and the BOSTID program for research relevant to this analysis. The data show that AID has committed about $8 million of research funding for investigations into these areas of interest since the AID-funded programs began in 1981. The duration of any single project may be from 1 to 4 years (73,255).

U.S. Department of Defense (DOD)

In fiscal year 1982, DOD obligated $233 million for biomedical research and development (383). Of this amount, $21 million (9 percent) was for research on about 30 infectious diseases. Almost $13.5 million of the latter amount was for research pertaining to the tropical diseases of interest in this analysis (140). That $13.5 million represents 64 percent of DOD's infectious disease research funding and 6 percent of DOD's total biomedical research funding.

Table 3-11 shows the levels of DOD funding for research pertaining to the six TDR diseases, diarrheal diseases, and arboviral diseases for fiscal years 1981, 1982, and 1983. DOD does only a small amount of research in ARIs. The figures in table 3-11 are for activities of the Army Medical Research and Development Command, which

Table 3-10.—Total AID Funds Committed Since 1981 for Research on Selected Diseases by the Program in Science and Technology Cooperation (PSTC) and the Board on Science and Technology for International Development (BOSTID)

	PSTC		BOSTID	
	Number of awards[a]	Amount (000s)	Number of awards[b]	Amount (000s)
Parasitic tropical diseases	21	$3,058	14	$1,575
Diarrheal diseases	6	851	0	0
ARIs and tuberculosis	2	271	4	598
Other	10	1,688	0	0
Total	39	$5,868	18	$2,173

[a] As of March 1984. The duration of each award may be from 1 to 4 years.
[b] As of January 1984. Awards are made three times each year.

SOURCES: **PSTC:** I. Asher, Office of the Science Advisor, U.S. Agency for International Development, Washington, DC, personal communication, March 1984.
BOSTID: National Research Council, Board on Science and Technology for International Development, "Grants Approved Through January 1984," mimeo, 1984.

Table 3-11.—DOD[a] Funding for Tropical Disease Research, Fiscal Years 1981-83

	Fiscal year 1981		Fiscal year 1982		Fiscal year 1983		Fiscal years 1981-83	
Disease	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent
TDR diseases[b]	$ 7,115	57%	$ 7,797	58%	$ 8,190	58%	$23,102	58%
Diarrheal diseases	1,558	12	1,635	12	1,717	12	4,910	12
Arboviral infections	3,815	31	4,004	30	4,204	30	12,023	30
Total by year	$12,488	100%	$13,436	100%	$14,111	100%	$40,035	100%

[a] These figures are for the U.S. Army, which manages the great majority of DOD research.
[b] Malaria, schistosomiasis, trypanosomiasis, filariasis, leishmaniasis, and leprosy.

SOURCE: M. Groves, U.S. Army Medical Research and Development Command, Ft. Detrick, MD, personal communication, March 1984.

manages almost all DOD tropical disease research efforts. The percentage of DOD funds for research in the specific categories of diseases remained almost constant from fiscal year 1981 to fiscal year 1983, with 58 percent of funds allocated to the six TDR diseases, 12 percent allocated to diarrheal diseases, and 30 percent allocated to arboviral diseases. The actual amounts available rose by approximately $1 million each year.

Table 3-12 shows the level and distribution of DOD funding for investigations in the six TDR diseases for fiscal years 1981 through 1983. As shown in that table, DOD conducts no research in filariasis or leprosy. About 58 percent of the DOD funding shown in table 3-12 is concerned with malaria; 19 percent with leishmaniasis; 17 percent with trypanosomiasis; and 7 percent with schistosomiasis.

Private U.S. Foundations

A number of private foundations in the United States fund activities in health, but do not report supporting biomedical research in the tropical diseases of interest in this analysis. These include the National Science Foundation, the Lasker Foundation, the Kellogg Foundation, the Ford Foundation, and the Carnegie Foundation. The paragraphs below describe the objectives and funding levels of the three U.S. foundations that do support these activities: the Edna McConnell Clark Foundation, the Rockefeller Foundation, and the MacArthur Foundation.

Edna McConnell Clark Foundation

The Edna McConnell Clark Foundation reports assets of $225 million in 1982 and makes grants of approximately $14 million annually (105). Grantmaking procedures suggest that applicants write a brief letter describing the program, which is reviewed by the appropriate program officer. If the request seems to fit the program's goals, more information and a proposal is requested. In reviewing proposals, the Clark Foundation's officers look for sound strategy and staff with the skills to accomplish work central to the aims of a particular program. Proposals are reviewed by trustees and a committee of outside experts and acted upon by the trustees.

The Clark Foundation makes grants in four program areas: 1) children in foster or institutional care, 2) jobs for the disadvantaged, 3) improvements in the criminal justice system, and 4) tropical disease research. Recently, the foundation made a decision to broaden support and include research on preventable causes of blindness. The tropical disease research program is currently concerned mainly with schistosomiasis, supporting research in immunology for vaccine development, the metabolism and biochemistry of schistosomes, and studies of the epidemiology of schistosomiasis.

The grants payable by the Clark Foundation's tropical disease program were $3,174,651 on September 30, 1980; $2,433,047 on the same date in 1981; and $2,238,369 in 1982 (106). These amounts

Table 3-12.—DOD[a] Funding for Research on the Six TDR Diseases, Fiscal Years 1981-83

Disease	Fiscal year 1981		Fiscal year 1982		Fiscal year 1983		Fiscal years 1981-83	
	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent
Malaria	$4,259	60%	$4,471	57%	$4,694	57%	$13,424	58%
Schistosomiasis[b]	270	4	611	8	644	8	1,525	7
Trypanosomiasis	1,224	17	1,285	16	1,350	16	3,859	17
Leishmaniasis	1,362	19	1,430	18	1,502	18	4,294	19
Filariasis	0	0	0	0	0	0	0	0
Leprosy	0	0	0	0	0	0	0	0
Total by year	$7,115	100%	$7,797	100%	$8,190	100%	$23,102	100%

[a] These figures are for the U.S. Army, which manages the great majority of DOD research.

[b] In fiscal year 1982 and fiscal year 1983, the Army and Navy schistosomiasis research programs were consolidated.

SOURCE: M. Groves, U.S. Army Medical and Research Development Command, Ft. Detrick, MD, personal communication, March 1984.

constitute about 30 percent, 25 percent, and 20 percent of total grants awarded by the foundation in each of these years.

Rockefeller Foundation

The Rockefeller Foundation's Health Sciences program began in 1977 and has three components: 1) the Great Neglected Diseases of Mankind, 2) the Health of Populations, and 3) Coping With Biomedical and Health Information. Since these components were put in place, the foundation has allocated about $12 million, $3 million, and $676,000 to the respective components (293).

The Rockefeller Foundation's Great Neglected Diseases program seeks to improve the knowledge, the means of treatment, and the control of these diseases by attracting outstanding scientists. The diseases included under the program are those such as malaria, schistosomiasis, hookworm, and diarrheal diseases. The objectives of the Rockefeller Foundation's efforts have been to establish and support research units of excellence, to establish collaboration in clinical investigation and field research among research institutions in developed and developing countries, and to maximize collaboration among these researchers through an annual meeting.

Funds for the Great Neglected Diseases program were $1.6 million in 1980, $1.9 million in 1981, and $1.9 million in 1982. By 1980, there were 14 research units established, 3 of which are located in the developing world, and collaboration had been established with 22 developing countries. Foundation funding for each research unit is $150,000 at most, and this support is about 30 percent of the support received by the units from all sources.

In addition to supporting these research units, the Rockefeller Foundation awards some grants related to tropical disease research to institutions not included under its program of institutional support. In 1981, such grants supported investigations in malaria, schistosomiasis, trypanosomiasis, and leprosy, as well as an investigation by the Center for Public Resources of ways in which the pharmaceutical industry might be encouraged to expand its role in improving health in developing countries.

MacArthur Foundation

In 1983, the board of the MacArthur Foundation decided to devote $20 million over the next 5 years to the support of centers of excellence to apply the techniques of modern biology to parasitic diseases (282). The MacArthur Foundation is particularly interested in supporting research by scientists who have not previously studied parasitic diseases and in fostering collaboration among scientific disciplines. Accordingly, it has invited proposals from a selected group of individuals and institutions and made its first awards under this program in late 1984. The MacArthur Foundation has also recently made a grant of $1 million to TDR.

Pharmaceutical Companies

Detailed information about the money spent by U.S. pharmaceutical companies to conduct biomedical research in tropical diseases is not available. Neither individual firms nor the industry publish comprehensive data about research expenditures because such information is considered proprietary. The discussion below summarizes relevant facts and conjectures about research on tropical diseases being undertaken by private companies.

Overview

The Pharmaceutical Manufacturers Association (PMA) reported in 1979 that its 132 U.S. members spent $1.3 billion each year on research. About one-fifth of the companies conduct 80 percent of the research and development in drugs. A PMA survey published in 1979 found that 21 companies had done, or were doing, research relevant to tropical diseases. PMA estimated that this research accounted for about 5 percent of the overall research and development effort of the industry (306).

Another report indicates that the U.S. pharmaceutical industry spends more than 50 percent of its net income on research and development (165). The report notes, however, that these activities are extremely concentrated within the industry. Only 14 of the 26 companies that spend more than $1 million a year on research and development actually spend at the industry average rate of 50 percent. The four most research-intensive companies account for 37 percent of the industry's research and development, but produce only 21 percent of industry sales. The next four most research-intensive companies account for 23 percent of the industry's research and development and 24 percent of its sales. The next 12 companies represent 33 percent of total research and development, but 47 percent of industry sales (165).

A survey of 15 research-oriented European pharmaceutical companies in 1977 found that 7 of these firms were engaged in tropical disease research. These companies allocated approximately $40 million to tropical disease research (387).

An analysis of the leading U.S. and European pharmaceutical companies in the area of research in tropical diseases suggests that seven companies spent a total of $22,327,000 on research in parasitology during fiscal year 1979 (73).

The data cited above should be reviewed in relation to the costs of developing new drugs. One study found that the average cost to a U.S.-owned pharmaceutical firm for developing a new chemical entity (NCE) to the point of marketing approval in the United States was $54 million in 1976 dollars. The average length of time from initiation of clinical testing to market approval for all NCEs approved in 1976 was more than 6 years. During the period 1963-76, approximately 900 NCEs were studied in humans by U.S.-owned firms. Of these 900, 20 (2 percent) were candidates primarily for tropical or parasitic diseases. These NCEs came from 11 U.S.-owned pharmaceutical firms (95).

Examples of Pharmaceutical Company Activities

In the past 25 years, a number of pharmaceutical companies have conducted biomedical research related to *malaria*, often in collaboration with the public sector. During the period 1960-69, for example, Parke-Davis invested about $16 million in antiparasite research, much of which was concerned with malaria (a disease for which Parke-Davis developed seven drugs). Furthermore, Parke-Davis reported in 1979 that, with AID support, it was engaged in studies of an immunological approach to malaria (20). Research in malaria by Roche led to the development of Fansidar, a prophylactic and chemotherapeutic drug marketed in 1970 (20). Since then, Roche has been collaborating with TDR and Walter Reed Army Institute of Research to conduct clinical trials of derivatives of the natural alkaloid quinine (20). A 1979 PMA survey found 14 U.S. companies and 4 of the 7 European firms surveyed were conducting research in malaria (306,387).

The PMA survey reported 11 American companies and 4 of the 7 European firms were engaged in *schistosomiasis* research (306,387). Pfizer's major work has been in schistosomiasis. Roche has been engaged in schistosomiasis research for more than 20 years and, after screening tests, has focused attention on two compounds, one of which has now been selected for clinical trials (20). Wellcome has worked on schistosomiasis for 30 years, but without commercial success (20). Bayer and E. Merck/Darmstadt began intensive research in schistosomiasis about 10 years ago (20).

Three of the 7 European firms surveyed by the PMA work in *trypanosomiasis* (387). Roche has given high priority to investigations into Chagas' disease (American trypanosomiasis), and reports that once the company succeeds, it will emphasize investigations pertaining to African trypanosomiasis (20). Bayer discovered the first effective drug against African trypanosomiasis in 1916, and after more than 30 years of research, Bayer introduced the first drug to treat the acute and chronic phases of Chagas' disease in 1972 (20). E. Merck/Darnstadt also has an active program in trypanosomiasis (20).

Research in *leishmaniasis* was stopped by many companies about 10 years ago because the market was small and a drug was available (20). However, TDR has provided some stimulus to new efforts, and six U.S. companies and two European companies are engaged in leishmaniasis research (306,387). Among these, Squibb was reported to be collaborating with TDR in supplying a compound to be tested in Africa (306). Burroughs-Wellcome is currently testing allopurinol, an existing drug used for gout, which was found to have antileishmanial activity.

Various companies have spent some $20 million on *filariasis* research, and there is as yet no satisfactory drug. Roche, Bayer, and Ciba-Geigy have been particularly active in this research effort, and Janssen has also been having good results (20). Seven members of the PMA reported research projects in filariasis, including Parke-Davis which was under contract to WHO for the synthesis of antifilarial drugs (306). Four of the seven European firms questioned in the PMA survey reported research in filariasis (387).

Leprosy is under investigation by two of the European firms and six members of the PMA (306,387). Ciba-Geigy and Lepetit have drugs on the market and are doing further research to try to reduce treatment costs by requiring a less frequent application (20).

Information about research into other diseases of interest to this analysis is not readily available. However, Lederle, Sterling, and Merck are reported to be conducting research in tuberculosis (306). The WHO program concerned with diarrheal diseases reports that during 1981-82, it collaborated with eight pharmaceutical companies, none of which were American (427).

The Rockefeller Foundation has sought to expand the role of the pharmaceutical industry in developing products for tropical diseases by making a series of grants to the Center for Public Resources. The center organized a task force of leaders from the pharmaceutical industry, the developing countries, academe, and assistance agencies following a 1979 conference at the Institute of Medicine. This task force designed a number of projects, but in 1981 and 1982, the pharmaceutical industry determined that it did not wish to match the Rockefeller Foundation's commitment to the cooperative effort and the individual projects, and the project was terminated. The Rockefeller Foundation points out that the outspoken support of Senators Jacob Javits, Richard Schweiker, and Edward Kennedy during the 1979 conference was not reflected in the political atmosphere of 1980, and suggests that industry cooperation may have been discouraged as a result (293).

TYPES OF RESEARCH FUNDED

Types of Tropical Diseases

Table 3-13 summarizes annual funding from WHO, TDR, and from U.S. Government and private foundation sources for research pertaining to the six TDR diseases, diarrheal diseases, ARIs, and arboviral diseases, for roughly the period 1979-81. Combined WHO/TDR and U.S. funding for biomedical research on these diseases was a little over $109 million per year. Less than $100 million came from U.S. sources.

Combined funding for research in the six TDR diseases—malaria, schistosomiasis, trypanosomiasis, leishmaniasis, filariasis, and leprosy—was almost $55 million, or about 50 percent of the total $109 million per year. Malaria research alone was funded at $22.8 million per year (21 percent of the total $109 million). TDR, AID's Bureau of Science and Technology, and DOD allocated a greater portion of their research funding for malaria research than for research in any of the other categories of tropical diseases considered in OTA's analysis. As shown in table 3-13, the distribution of combined annual funding for biomedical research on the other TDR diseases was as follows: schistosomiasis, $9.2 million (8 percent of the total $109 million); trypanosomiasis, $9 million (8 percent); leishmaniasis, $6.3 million (6 percent); filariasis $3.7 million (3 percent); and leprosy, $3.8 million (4 percent).

Research in diarrheal diseases was funded at an annual level of $14.5 million (or about 13 percent of the total $109 million per year); research in ARIs was funded at $20.7 million (19 percent of the total); and research in arboviral diseases was funded at $7.5 million (7 percent of the total). Combined annual funding for research that is related to the diseases of interest in this analysis but for which data were not available in sufficient detail to allocate the funding by disease (the "unspecified category in table 3-13) amounted to $11.5 million (11 percent of the total).

Table 3-13.—Summary of Annual Funding by U.S. Sources[a] and WHO/TDR Sources[b] for Biomedical Research on Selected Tropical Diseases, Various Years[c]

Disease	U.S. sources[b] (000s)	WHO/TDR[c] (000s)	Combined WHO/TDR and U.S. sources: Amount (000s)	Combined WHO/TDR and U.S. sources: Percent
Malaria	$19,354	$ 3,435	$ 22,789	21%
Schistosomiasis	8,136	1,044	9,180	8
Trypanosomiasis	6,716	2,335	9,051	8
Leishmaniasis	5,707	614	6,321	6
Filariasis	2,137	1,548	3,685	3
Leprosy	1,546	2,299	3,845	4
Subtotal	$43,596	$11,275	$ 54,871	50%
Diarrheal diseases	$14,081	$ 417	$ 14,498	13%
Acute respiratory infections	20,301	381	20,682	19
Arboviral diseases	7,482	NA	7,482	7
Subtotal	$41,864	$ 798	$ 42,662	39%
Unspecified	$10,359	$ 1,109	$ 11,468	11%
Total by source	$95,819	$13,182	$109,001	100%
Percent of combined funding amount	88%	12%		

[a] This category includes U.S. Government agencies and private foundations shown in previous tables.

[b] This category includes TDR and WHO funds as shown in tables 3-1, 3-2, and 3-3.

[c] Data are presented for most recent years available, as noted on previous tables.

SOURCE: Compiled from data presented in previous tables in this chapter and the 1981 reports of the Rockefeller Foundation and the Edna McConnell Clark Foundation.

These data are affected by the fact that not all agencies define diarrheal diseases and ARIs as tropical diseases. Research funds for these diseases from NIAID sources other than those that fall under tropical medicine are included, but it is likely that WHO and other institutes of NIH, in particular, fund biomedical research in these diseases which is not included in this analysis.

Furthermore, information about the contribution of pharmaceutical companies to research in these diseases is not available. Although the PMA suggests that tropical disease research receives some 5 percent of the $1.3 billion spent by all U.S. industry on biomedical research and development, this figure cannot be substantiated.

Types of Research Objectives

Recently, AID attempted to classify TDR research projects according to four research objectives: diagnostic methods, chemotherapy, immunology and vaccination, and vector control (108). AID compiled a matrix of research projects by looking at a list of TDR project titles and, using the opinion of a small number of experienced scientists, allocated each research project to one disease category and one research objective category. The matrix that was developed by AID cannot be construed as comprehensive or definitive, but it does give some indication of the relative attention paid to each objective as research funds were allocated among diseases and disease types.

Table 3-14 is an expansion of AID's matrix for the six TDR diseases. The figures in table 3-14 include projects funded by NIAID, AID, DOD, the Rockefeller Foundation, and the Edna McConnell Clark Foundation, as well as those funded by TDR.

In the data presented in table 3-14, the research projects included under TDR include relevant projects funded under both the disease-specific and transdisease components of TDR's Research and Development program area. Thus, in table 3-14 (unlike in table 3-1 concerned with TDR as a funding source), a research project funded under one of the four transdisease components of the program area (i.e., biomedical sciences, vector control and biology, epidemiology, or social and economic research) was included if it seemed to relate directly to one of the six TDR diseases.

Table 3-14.—Distribution of Funding for Biomedical Research on the Six TDR Diseases[a] by Research Objective, 1981

Disease	Diagnostic methods		Chemotherapy		Immunology/vaccination		Vector control		Other		Total	
	Number of projects	Amount (000s)	Number of projects	Amount (000s)	Number of projects	Amount (000s)	Number of projects	Amount (000s)	Number of projects	Amount (000s)	Number of projects	Amount (000s)
Malaria	2	$ 118	47	$3,819	67	$ 8,128	16	$1,166	27	$ 2,797	159	$16,028
Schistosomiasis	6	310	35	1,748	48	2,800	15	446	31	2,400	135	7,704
Trypanosomiasis	4	245	15	1,027	37	2,397	6	156	40	3,145	102	6,970
Leishmaniasis	6	409	11	728	11	917	4	106	13	1,532	45	3,692
Filariasis	3	156	18	1,237	15	971	3	136	10	716	49	3,216
Leprosy	5	164	22	785	19	693	NA	NA	15	1,052	61	2,694
Total by research objective	26	$1,402	148	$9,344	197	$15,906	44	$2,010	136	$11,642	551	$40,304
Percent of total number of projects	5%		27%		36%		8%		25%		100%	
Percent of total funding		3%		23%		39%		5%		29%		100%

[a] Numbers of projects and funding amounts are totals for research funded by the following: TDR, NIAID, AID, DOD, the Rockefeller Foundation, and the Edna McConnell Clark Foundation.

SOURCES: J. Elliot and P. Contacos, "A Profile of Selected Biomedical Research Efforts into Diseases of Major Public Health Importance to People of Developing Countries," typescript prepared for the U.S. Agency for International Development, Washington, DC, November 1982; M. Groves, U.S. Army Medical Research and Development Command, Ft. Detrick, MD, personal communication, March 1984; J. Nutter, Chief, Office of Program Planning and Evaluation, NIAID, personal communication, April 1984; and J. Erickson, Division Chief, Office of Health, U.S. Agency for International Development, personal communication, March 1984.

Although the data in table 3-14 are not comprehensive and are only an indication of research objectives, they do show that immunology/vaccination was the objective of 39 percent of the total $40.3 million funding and 35 percent of the 551 projects. Research in malaria immunology and vaccination accounted for $8.1 million (20 percent of the total funds shown). Research in chemotherapy amounted to $9.3 million (27 percent of the total); vector control, $2 million (8 percent); and diagnostic methods, $1.4 million (3 percent). Biomedical research in other aspects of the TDR diseases amounted to $11.6 million, which is 29 percent of the total $40.3 million.

Table 3-15, presenting data on NIAID-funded research concerned with diarrheal and enteric infections and ARIs, shows that in fiscal year 1981, research projects with the objective of immunology/vaccination accounted for over $9 million, or 39 percent of the $23.2 million funding NIAID provided. Research projects with objectives other than diagnostic methods, chemotherapy, or immunology and vaccination accounted for $12.8 million, or 55 percent of the total $23.2 million.

RECIPIENTS OF RESEARCH FUNDING

The organizations receiving funds for biomedical research in tropical diseases are predominantly universities and research institutes. Table 3-16 presents information about the distribution of extramural biomedical research funds from various sources to institutions in the United States, in other industrialized countries, and in less developed countries.

Table 3-16 suggests that about one-third of TDR biomedical research funding in 1981 was awarded to institutions in the United States. The *Country Profile: USA*, published by TDR, reports that between 1975 and mid-1982, $15,103,228 (20 percent) of the $77.3 million obligated by the program was in support of research and training by U.S. institutions. During this same period, the United States contributed $15,372,912 to TDR (352).

CDD, the WHO program in diarrheal diseases, awarded $214,000, or one-quarter of its biomedical research funds, to U.S. institutions during 1982 (see table 3-16). In 1982, the United States contributed less than $80,000 to CDD, about 1 percent of the total CDD budget (427).

NIAID research in tropical medicine and international health is concentrated at U.S. institutions (see table 3-16), though these institutions often work in close collaboration with scientists in the developing world. In addition, NIAID has a role in bilateral programs for scientific exchange and collaboration with Egypt, Israel, and India, which are funded by other agencies. On occasion, NIAID makes grant and contract awards to institutes outside the United States, usually in other industrialized countries. The distribution of NIAID funding for intramural and extramural (U.S. and foreign) research in tropical diseases during fiscal years 1981, 1982, and 1983 is shown in table 3-17.

In fiscal year 1983, AID's Bureau of Science and Technology allocated almost three-quarters of its biomedical research funds to U.S. institutions (see table 3-16) (110). Many of these institutions collaborate with organizations in less developed countries. The remaining biomedical research funding is in the form of support to the International Center for Diarrheal Disease Research in Bangladesh. AID's Research Grants Program administered by the Science Advisor's Office and by BOSTID has allocated 45 percent of its resources to American institutions (11,255). The BOSTID portion of this program makes grants only to institutions in developing countries.

The DOD biomedical research program allocates about three-quarters of its extramural funds to organizations in the United States (see table 3-16). A further 18 percent is allocated to organizations in less developed countries and 5 percent to organizations in industrialized countries other

Table 3-15.—NIAID Funding for Biomedical Research on Diarrheal and Enteric Infections and ARIs by Research Objective, Fiscal Year 1981

	Diagnostic methods		Chemotherapy		Immunology/ vaccination		Other		Total
	Number of projects	Amount (000s)	Number of projects	Amount (000s)	Number of projects	Amount (000s)	Number of projects	Amount (000s)	Amount (000s)
Diarrheal and enteric infections	0	$ 0	0	$ 0	17	$1,657	51	$ 5,299	$ 6,956
ARIs	7	749	6	487	48	7,443	76	7,582	16,261
Total by research objective	7	$749	6	$487	65	$9,100	127	$12,881	$23,217
Percent of total amount for all research objectives		3%		2%		39%		55%	100%

SOURCE: J. E. Nutter, Chief, Office of Program Planning and Evaluation, NIAID, National Institutes of Health, Bethesda, MD, personal communication, April 1984.

Table 3-16.—Distribution of Extramural Biomedical Research Funds by Various Funding Sources to Recipient Organizations in the United States, Other Industrialized Countries, and Less Developed Countries, Various Years

	United States		Other industrialized countries		Less developed countries		Total
Funding source	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)
TDR (1981)	$ 4,003	32%	$4,313	34%	$ 4,386	35%	$12,702
WHO/CDD (1982)	214	25	205	24	438	51	857
NIAID (fiscal year 1982)[a]	24,346	96	1,039	4	0	0	25,385
AID/Bureau of Science and Technology[b] (fiscal year 1983)	5,987	73	0	0	2,217	27	8,204
AID/Science Advisor[c]	3,608	45	0	0	4,433	55	8,041
DOD (fiscal year 1983)	3,984	76	285	5	952	18	5,221
Edna McConnell Clark Foundation (1981)	1,667	77	436	20	76	3	2,179
Rockefeller Foundation (1981)	995	52	579	30	345	18	1,919
Total by recipient country	$44,804		$6,857		$12,847		$64,508
Percent of total for all recipient countries		69%		11%		20%	100%

[a] Investigations undertaken by U.S. institutions and funded by NIAID are often carried out in close collaboration with institutions in less developed countries. These data include funds for training in biomedical research and exclude intramural research projects. The data are for NIAID's tropical disease research only.

[b] Does not include $3 million contribution to TDR.

[c] Research funds committed as of March 1984.

SOURCES: Derived from: **TDR, Rockefeller Foundation, Clark Foundation:** V. Elliott and P. Contacos, "A Profile of Selected Biomedical Reearch Efforts Into Diseases of Major Public Health Importance to People of Developing Countries," typescript, prepared for the U.S. Agency for International Development, Washington, DC, November 1982. **WHO/CDD:** World Health Organization, *Program for Control of Diarrheal Diseases*, DCC/TAG/84.2A (Geneva: WHO, 1984). **NIAID:** U.S. Department of Health and Human Services, National Institutes of Health, "International Cooperation by the National Institute of Allergy and Infectious Diseases, FY 1982," mimeo, Bethesda, MD, no date. **AID:** J. Erickson, Division Chief, Office of Health, U.S. Agency for International Development, Washington, DC, personal communication, March 1984. **AID:** I. Asher, Office of the Science Advisor, U.S. Agency for International Development, Washington, DC, personal communication, March 1984. **DOD:** M. Groves, U.S. Army Medical Research and Development Command, Ft. Detrick, MD, personal communication, March 1984.

Table 3-17.—NIAID Funding for Tropical Medicine and International Health by Type of Recipient, Fiscal Years 1981-83

	Fiscal year 1981		Fiscal year 1982		Fiscal year 1983		Fiscal years 1981-83	
	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent
Intramural	$ 6,553	21%	$ 9,472	27%	$ 7,856	23%	$ 23,881	24%
Extramural U.S.[a]	23,542	75	24,346	70	25,576	74	73,464	73
Extramural foreign[b]	1,155	4	1,039	3	963	3	3,157	3
Total[c]	$31,250	100%	$34,857	100%	$34,395	100%	$100,502	100%

[a] The extramural program includes grants and contracts.

[b] Foreign recipients of extramural grants or contracts are predominantly organizations in other industrialized countries.

[c] These figures include funds for training in biomedical research.

SOURCE: U.S. Department of Health and Human Services, National Institutes of Health, "International Cooperation by the National Institute of Allergy and Infectious Diseases, FY 1981, FY 1982, FY 1983," mimeos, Bethesda, MD, no dates.

than the United States. The distribution of DOD funding, both U.S. and foreign, in fiscal years 1981, 1982, and 1983 is shown in table 3-18.

In 1981, the majority of research funds of both the Rockefeller Foundation and the Edna McConnell Clark Foundation was awarded to U.S. institutions (see table 3-16). The Rockefeller Foundation awarded 52 percent of grants in the United States, 30 percent to other industrialized countries, and 18 percent to less developed countries (293). The comparable figures for the Edna McConnell Clark Foundation are 77 percent, 20 percent, and 3 percent (105).

Institutions in the United States received $44.8 million (69 percent) of biomedical research funds from the funding sources identified in table 3-16. Institutions located in other industrialized countries received $6.7 million (11 percent); and those in less developed countries received $12.8 million (20 percent).

Table 3-18.—DOD[a] Funding for Biomedical Research on Tropical Diseases by Type of Recipient, Fiscal Years 1981-83

	Fiscal year 1981		Fiscal year 1982		Fiscal year 1983		Fiscal years 1981-83	
	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent	Amount (000s)	Percent
Intramural	$ 7,867	63%	$ 8,431	63%	$ 8,890	63%	$25,188	63
Extramural U.S.	3,244	26	3,554	26	2,984	28	10,782	27
Extramural foreign[b]	1,377	11	1,450	11%	1,237	9	4,064	10
Total by year	$12,488	100%	$13,436	100%	$14,111	100%	$40,034	100%

[a] These figures are for the U.S. Army, which manages the great majority of DOD research.

[b] About 21 percent of extramural funding of foreign organizations is received by organizations in other industrialized countries, the remainder by organizations in less developed countries.

SOURCE: M. Groves, U.S. Army Medical Research and Development Command, Ft. Detrick, MD, personal communication, March 1984.

SUMMARY

Several departments and agencies of the U.S. Government support biomedical research on tropical diseases. The important players are DHHS, AID, and DOD. The U.S. Government also supports international programs in tropical disease research. Of greatest consequence are TDR and WHO's various research programs. A small number of U.S. foundations support research in tropical diseases. The Rockefeller Foundation and the Edna McConnell Clark Foundation have a long history of involvement in this field, and the MacArthur Foundation has become active more recently. A handful of pharmaceutical companies also invest in research to develop products for tropical diseases, but funding levels are undocumented.

Actual dollar amounts for funding of tropical disease research are difficult to assemble for a variety of reasons. The figures presented in this report represent the best efforts of the public and private bodies to provide this information, and OTA believes that the totals are sufficiently accurate to give a rough estimate of U.S. spending for tropical disease research. OTA estimates that U.S. public and private organizations (excluding pharmaceutical companies) have spent somewhat less than $100 million per year on tropical disease research. This figure includes contributions to international programs.

A high proportion of U.S. tropical disease research funds is awarded to U.S. investigators. About 96 percent of NIAID extramural research funds for this purpose goes to Americans. Perhaps more surprising, U.S. research institutions and investigators have been awarded at least as much as the U.S. Government has contributed to TDR and the WHO programs. Only one program—the AID-supported Research Grants Program administered by the National Academy of Sciences' BOSTID—is specifically designed to make grants only to institutions in developing countries.

4.

Description of Selected Tropical Diseases

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4.
Description of Selected Tropical Diseases

INTRODUCTION

This chapter describes the major tropical diseases that are discussed in this report. The diseases considered are a rather diverse group.

Representing the diseases traditionally considered "tropical" in this report are malaria, schistosomiasis, trypanosomiasis, leishmaniasis, filariasis, and leprosy. These are the six diseases singled out for attention by the Special Program for Research and Training in Tropical Diseases (TDR), which is sponsored jointly by the U.N. Development Program, the World Bank, and the World Health Organization (WHO). The six TDR diseases together affect between 700 million and 800 million people worldwide. Malaria, schistosomiasis, and filariasis each affects more than 200 million people.

Other diseases discussed in this report—tuberculosis, diarrheal diseases, acute respiratory infections (ARIs), and arboviral infections—occur in nontropical countries as well as tropical ones. The toll these diseases take in developing countries is much greater than the toll they take in the developed world because of developing countries' higher incidence rates and poorer diagnostic and therapeutic methods.

Tuberculosis was once a public health problem worldwide. It now persists mainly in developing countries, where it infects large numbers of people.

Diarrheal diseases and ARIs are the leading causes of death among infants and children in developing countries. In countries where the infant mortality rate is more than 100 per 1,000 live births, at least one-third of infant deaths are from diarrheal diseases.

Arboviral infections have a worldwide distribution, but are concentrated in the tropics. With no specific treatments available and a vaccine against only two (yellow fever and Japanese encephalitis), arboviral infections are a potential threat to the U.S. population.

These are far from the only health problems in tropical developing countries. In addition to being affected by the often debilitating infectious diseases discussed here, people in the developing world are increasingly affected by the chronic diseases that cause so much sickness and death in the developed world: heart disease, cancer, stroke, and diabetes, for instance. Ironically, these chronic diseases increase in incidence as life expectancy increases and as the probability of dying from infectious diseases decreases.

This chapter provides basic information about each of the diseases considered in the later chapters of this report. Chapter 5 discusses strategies for controlling tropical diseases, and chapters 6 through 9 examine the status of disease control measures: vector control technologies, immunization technologies, diagnostic technologies, and therapeutic technologies. Table 4-1 summarizes some basic information about each disease and guides the reader to the relevant sections, for each disease, in the remainder of the report.

MALARIA

Aspects of Natural History

In 1940, the Nobel Laureate Sir Macfarlane Burnet wrote (37):

> If we take as our standard of importance the greatest harm to the greatest number, then there is no question that malaria is the most important of all infectious diseases.

His statement still holds true. Malaria is one of the most studied of all tropical diseases. The disease is caused by various species of the protozoan genus *Plasmodium*. Malaria parasites have a com-

Table 4-1.—Location in This Report of Information About Selected Tropical Diseases

Disease/ type of infection	Causative agent	Vector or mode of transmission	Map (or distribution)	Disease description	Status of:		
					Vaccines	Diagnostics	Therapeutics
Malaria			63	59	134,225	163	182
Protozoal	*Plasmodium* spp.	*Anopheles* spp.					
Schistosomiasis			68	65	137	164	185
Helminthic	*Schistosoma* spp.	Various snails					
Trypanosomiasis			71,74	67	138	165	186
Protozoal							
African sleeping sickness							
(West)	*Trypanosoma brucei gambiense*	*Glossina* spp. (tsetse flies)					
(East)	*T. b. rhodesiense*						
Chagas' disease	*T. cruzi*	Reduviid bugs ("kissing bugs")					
Leishmaniasis			77	74	140	167	187
Protozoal	*Leishmania* spp.	*Phlebotomus* spp. (sandflies)					
Filariasis			81	78	140	168	188
Helminthic	*Wuchereria bancrofti* *Brugia malayi*	*Anopheles*, and others (mosquitoes)					
Onchocerciasis	*Onchocerca volvulus*	*Simulium* spp. (blackflies)					
Leprosy			83	80	140	169	188
Bacterial	*Mycobacterium leprae*	Suspect airborne					
Tuberculosis			Worldwide	84	142	170	189
Bacterial	*Mycobacterium tuberculosis*	Airborne droplets					
Diarrheal and enteric diseases			Worldwide	85	142	172	190,201
Viral	Various viruses	All by fecal contamination of food and/or water					
Bacterial	Various bacteria, e.g., *Vibrio cholerae, Escherichia coli*						
Protozoal	*Entameba histolytica, Giardia lamblia*						
Helminthic	Various helminths, e.g., *Ascaris, Necator*						
Acute respiratory infections (ARIs)			Worldwide	88	144	173	193
Viral	Various viruses	All by airborne droplets					
Bacterial	Various bacteria						
Chlamydial	*Chlamydia* spp.						
Mycoplasmal	*Mycoplasma* spp.						
Arboviral infections			Worldwide (e.g., yellow fever [p. 94]; dengue fever [p. 95])	91	148	176	196
Viral	Various viruses	Various arthropods					

SOURCE: Office of Technology Assessment, 1985.

plex life cycle, alternating between vertebrate hosts and mosquito vectors of the genus *Anopheles*.

Four species of *Plasmodium* cause malaria in humans: *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. Other species of *Plasmodium* infect a wide variety of other vertebrates.

The four species that infect humans have somewhat different clinical effects. *P. falciparum* can cause severe anemia, kidney failure, and brain damage and is often fatal, especially in children. *P. vivax* and *P. ovale* infections are seldom fatal, but relapses of the symptoms (chills, high fever, nausea, headache, etc.) can occur periodically for up to 3 years. *P. malariae* infections can persist in the blood for years without causing any symptoms.

Life Cycle

All species of *Plasmodium* progress through a similar life cycle, though each species differs in some of the details. All human malaria infection begins with the bite of an infected female *Anopheles* mosquito (see fig. 4-1). As the mosquito ingests a blood meal to nourish her eggs, she incidentally injects saliva containing plasmodial sporozoites (which have been clustered in the mosquito's salivary glands) into the human bloodstream.

Within about an hour, the threadlike sporozoites leave the bloodstream and move to the human liver. Over the next week or two, depending on the species of *Plasmodium*, each sporozoite that has invaded a liver cell becomes a schizont, a developmental stage that contains thousands of merozoites, the next stage in the life cycle of the parasite. When the schizont matures, it ruptures out of the infected liver cell and discharges merozoites into the human host's bloodstream. In *P. vivax* and *P. ovale* malaria, some sporozoites, instead of developing into shizonts, become hypnozoites, forms that can remain dormant in the liver for months or years before they start to proliferate.

Merozoites invade red blood cells (erythrocytes) and there undergo a second round of asexual reproduction, similar to that in the liver. In 2 or 3 days, the merozoites develop into trophozoites, then into a second dividing schizont form. When the schizonts mature, they rupture and release another round of merozoites, perpetuating the cycle of infection. It is at the time of this rupture that clinical symptoms of malaria appear. The cycle repeats every 2 or 3 days, depending on the species of *Plasmodium*.

Some merozoites, instead of developing into schizonts, differentiate into sexual forms, gametocytes. Mature gametocytes remain in the host's red blood cells, and can be ingested by female *Anopheles* when they bite. Gametocytes develop further into male and female gametes, which undergo sexual reproduction in the mosquito's gut. Eventually a new generation of sporozoites develops in the mosquito and migrates to the mosquito's salivary glands, ready to infect another human.

Incidence and Prevalence

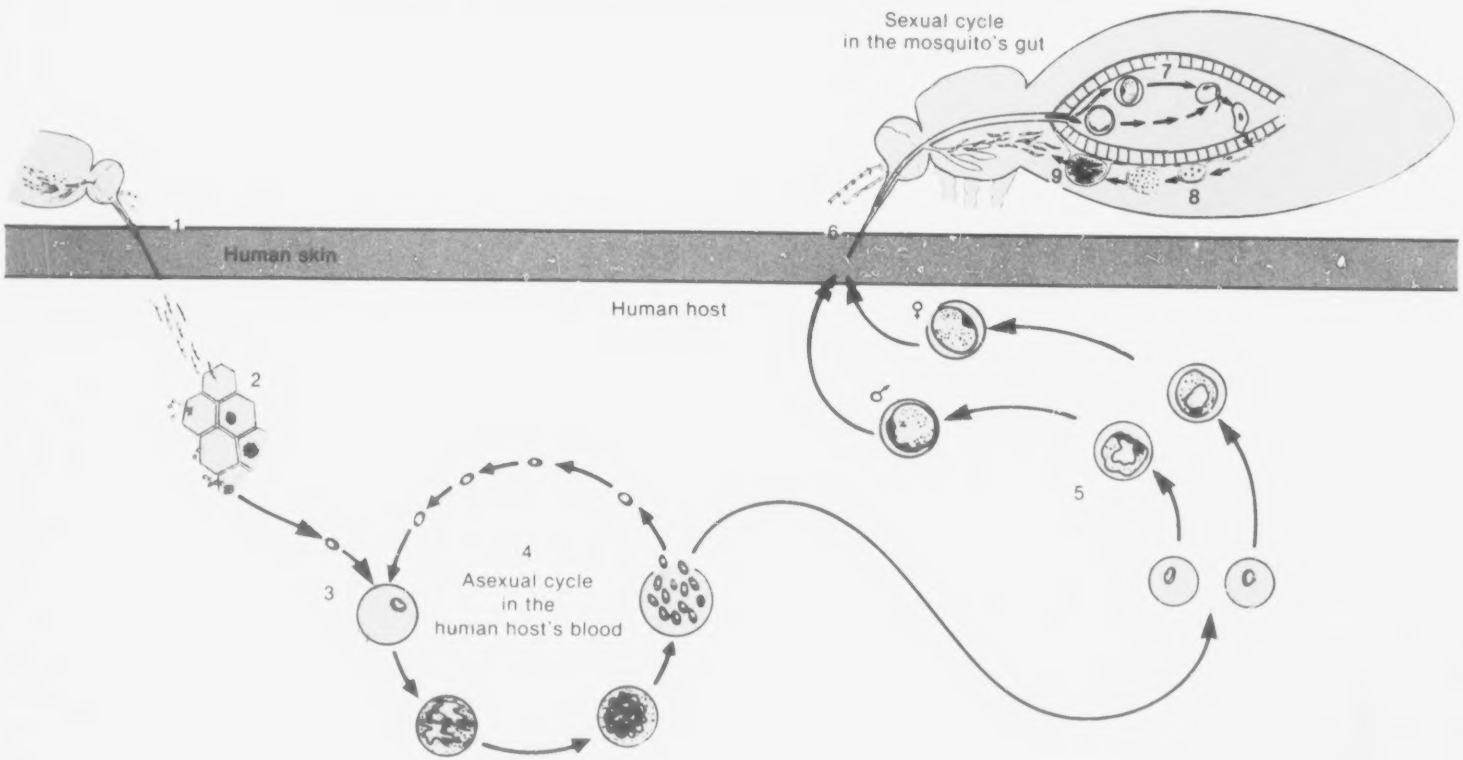
Malaria is one of the most widespread and destructive of diseases, having doubled in world prevalence in the last decade (430). Worldwide, an estimated 250 million to 300 million cases of malaria occur each year. In tropical Africa alone, an estimated 160 million to 200 million people are infected every year, and 1 million people die, mostly infants and small children (193).

About 1.5 billion people live in areas of the world where the risk of malaria ranges from moderate to high (see fig. 4-2). The countries of highest malaria incidence are Haiti, Guatemala, Honduras, El Salvador, Colombia, Bolivia, Brazil, India, Sri Lanka, Pakistan, and many parts of Africa and Asia. In Europe, the Caribbean, North America, and parts of South America, and Australia, the gains against malaria brought about by the WHO eradication program (see discussion below) have been maintained. However, developed nations such as England and the United States have experienced an increase in the number of imported malaria cases.

Malaria Control

Before World War II, approximately two-thirds of the world's population was at risk for malaria. DDT (dichloro-diphenyl-trichloro-ethane) was initially highly successful at controlling the *Anopheles*

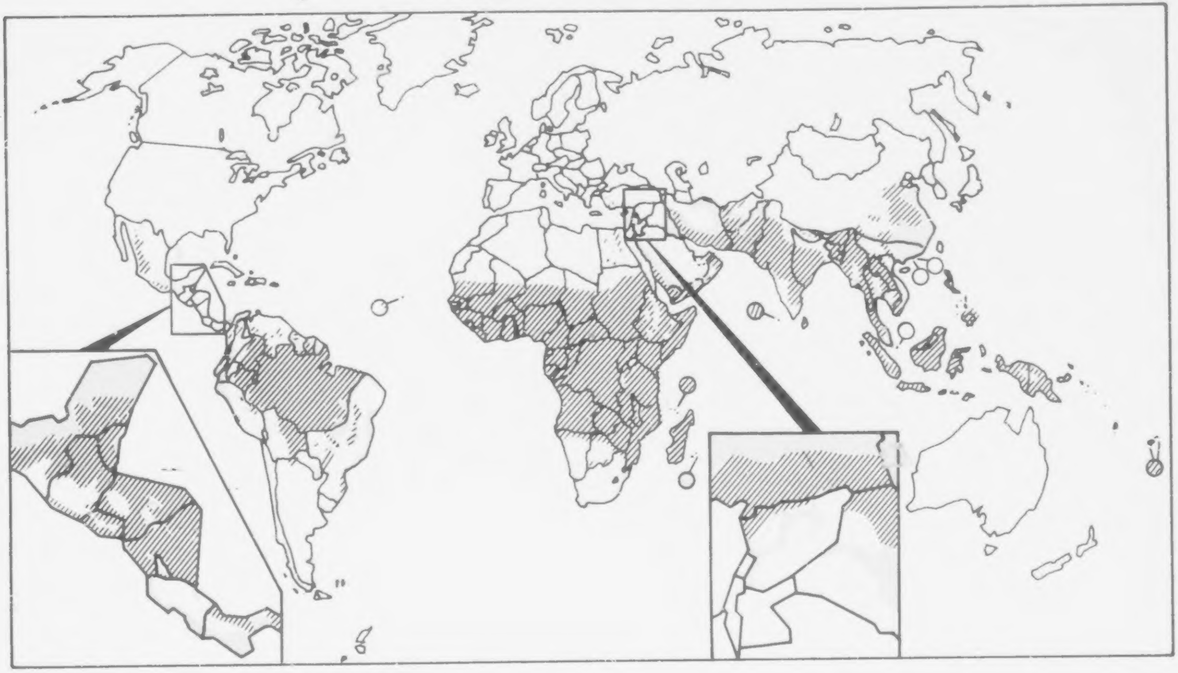
Figure 4-1.—Generalized Life Cycle of *Plasmodium*, the Cause of Malaria



1. Female *Anopheles* mosquito bites a human, injecting malaria sporozoites.
2. Sporozoites quickly move to the human host's liver cells, where they undergo asexual reproduction.
3. Merozoites released from liver cells enter the human host's bloodstream and invade red blood cells.
4. Merozoites undergo asexual reproduction in the human host's red blood cells, releasing more merozoites to infect more red blood cells. This cycle may be repeated many times in chronic infections.
5. Some merozoites differentiate to become gametocytes.
6. Gametocytes are picked up by a female *Anopheles* mosquito, in which the life cycle of the malaria parasite is completed.
7. Gametocytes develop further into male and female gametes, then fuse to form zygotes within the mosquito's gut.
8. The developing zygote burrows into cell lining of mosquito gut and develops into a cyst containing thousands of sporozoites.
9. Sporozoites are released into the mosquito's body cavity and migrate to the salivary glands, where they are in position to enter a human when the mosquito bites.

SOURCE: Adapted from H. W. Brown and F. A. Neva, *Basic Clinical Parasitology* (East Norwalk, CT: Appleton-Century-Crofts, 1983).

Figure 4-2.—Geographic Distribution of Malaria as of 1982



Key:

☐ Areas in which malaria has disappeared, been eradicated, or never existed

☐ Areas with limited risk

▨ Areas where malaria transmission occurs

SOURCE: U.S. Department of Health and Human Services, Centers for Disease Control, *Health Information for International Travel*, HHS Publication No. (CDC)840-8280 (Washington, DC: U.S. Government Printing Office, 1984).

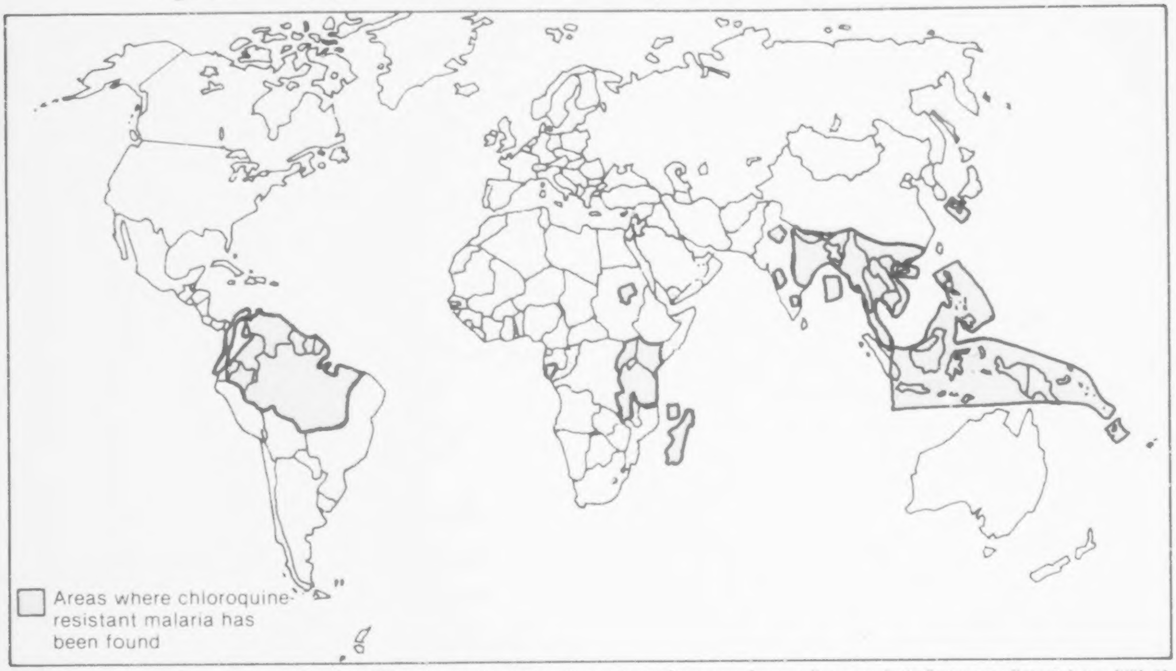
mosquito vector of malaria, however, and because of this, WHO began a worldwide malaria eradication program in the late 1950s. Large-scale spraying efforts kept the mosquito population down, eliminating the risk of disease for some 400 million people, mostly in temperate regions.

By the mid-1960s, malaria had been eliminated from nearly all of Europe, most of the Asian part of the U.S.S.R., several countries of the Near East, most of North America including all of the United States, most of the Caribbean, large areas of the northern and southern parts of South America, Australia, Japan, Singapore, Korea, and Taiwan. Eighty percent of the originally malarious area was almost free of malaria.

By 1969, however, there had been little additional progress. The worldwide eradication campaign failed for several reasons. Increasing mosquito resistance to DDT and *P. falciparum* resistance to chloroquine, the most widely used malaria drug, were two of the most important (33). Other factors in the increased incidence of malaria were behavioral changes in the mosquito vector species and the migration of workers who unintentionally brought the vectors with them to new areas.

While efforts to keep the *Anopheles* vector under control have been successful in some areas, the cost of applying traditional malaria interventions has greatly restricted the ability of developing countries to protect the populations at risk. Furthermore, few efforts to slow the spread of drug-resistant malaria have worked. Resistance of *P. falciparum* to chloroquine has been present in Panama, parts of some South American countries, India, Southeast Asia, Indonesia, China, the Republic of the Philippines, and other Pacific Islands for several years (see fig. 4-3). In Latin

Figure 4-3.—Geographic Distribution of Chloroquine-Resistant Malaria as of 1983



SOURCES: U.N. Development Program/World Bank/WHO, Special Program for Research and Training in Tropical Diseases, *Sixth Programme Report, 1 July 1981–31 December 1982* (Geneva: WHO, 1983).
U.S. Department of Health and Human Services, Centers for Disease Control, *Health Information for International Travel*, HHS Publication No. (CDC)840-8280 (Washington, DC: U.S. Government Printing Office, 1984).

America, resistant strains of the organism are appearing in most parts of Bolivia, Venezuela, French Guyana, and northern Peru, but serious drug-resistance has still not moved north of the Panama Canal. There is growing evidence of resistance in east Africa, which includes Kenya, Tanzania, eastern Zaire, Burundi, Uganda, Rwanda, Malawi, Zambia, northern Sudan, Madagascar, and the Comoro Islands (376).

Resistance to chloroquine is not the only problem. Some malaria parasites have also developed resistance to newer drugs. Regular monitoring of local parasite strains is necessary as an indicator of therapeutic changes that may be needed, both in type of drug and dose. Renewed efforts to develop new drugs are producing results, but moving new drugs from laboratory screening through animal testing to human trials takes years.

Recent Progress

Since 1976, with the development of methods allowing for the continuous cultivation of malaria parasites in the laboratory, research on the immunology of malaria has moved ahead considerably. All life cycle stages of the malaria parasite can now be grown in culture. An important recent discovery is that the proteins on the surface of the parasite (surface antigens) vary not only among the species of *Plasmodium* but also among strains of a single species. Perhaps of even greater interest is the finding that malaria parasites can change their surface antigens during the course of an infection. The human (or animal) host, in turn, must play "catch-up" in order to destroy the new forms of the parasite. This finding has direct implications for the development of vaccines and diagnostic tests (231).

Basic malaria research has advanced considerably. Metabolic studies have identified parasite-specific enzyme pathways that can be exploited to kill the malaria parasite without harming the human host. Membrane research has revealed how the parasite finds, attaches to, and invades the human host's red blood cells, yielding important clues for drug therapy and vaccine research. Recent clinical studies have suggested better ways of preventing and treating cerebral malaria, an often fatal complication of severe malaria infection.

A vaccine against the sporozoite stage of malaria may be available for testing by 1986. Vaccines against the merozoite and the gamete stages are behind this in development. If animal testing in humans confirms the feasibility of immunization against the malaria parasite, extensive human and field trials will be required before the vaccine can be widely used. Furthermore, given the difficulties that have plagued other disease immunization campaigns there is some debate about the usefulness of a malaria vaccine (see *Case Study B: The Development of a Malaria Vaccine* for a more complete discussion).

Research Needs

Field- and community-based studies are needed to assess the impact of antimalarial interventions. The emergence of insecticide-resistant mosquito vectors of malaria has seriously handicapped current malaria control efforts, making studies of the ecological impact of future interventions critically important. The effect of antimalarial activities on the actual immunity levels of populations needs clarification. Studies in the past have clearly documented the immediate impact of antimalarial projects on morbidity and mortality, but not the long-term consequences when projects cease or fail. Other studies are needed to evaluate the importance of sociological and human behavioral factors and the usefulness of health education, community self-help, and volunteer collaborators.

SCHISTOSOMIASIS

Aspects of Natural History

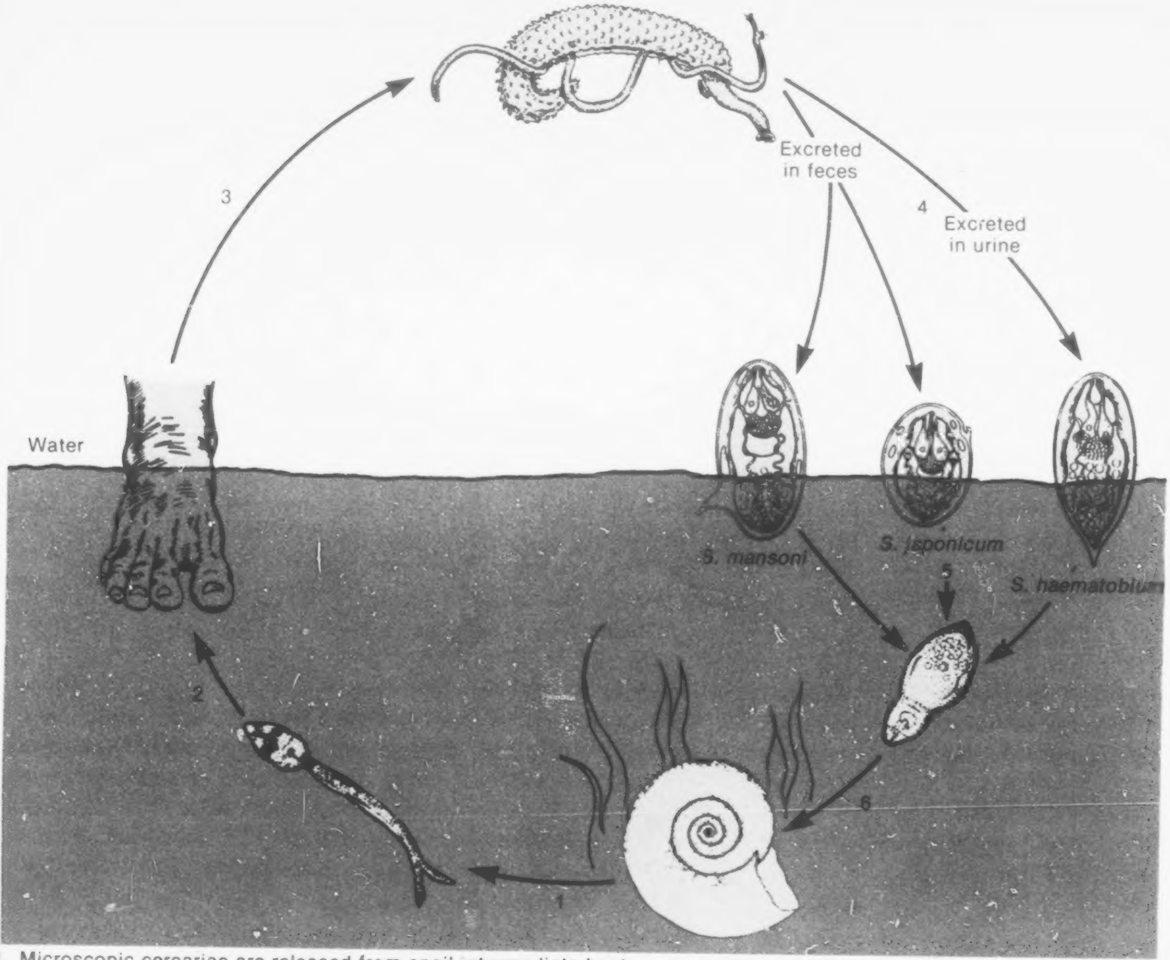
Schistosomiasis is a debilitating disease caused by trematode worms of the genus *Schistosoma*. There are three major species affecting humans, *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*, all of which originated in the Old World but now occur worldwide. *S. mansoni* originated in Africa, but now has become established in the Americas. A fourth species, *S. mekongi*, was discovered in the late 1960s and is endemic in areas of Southeast Asia.

Life Cycle

The adult parasitic worms that cause schistosomiasis live in pairs in the human host's bloodstream, sometimes for many years. Eggs (from a few hundred to several thousand per day) are produced by the female schistosome and are deposited in the host's blood vessels. The eggs escape into the host's bowel (*S. mansoni*, *S. japonicum*) or bladder (*S. haematobium)*, and then are excreted in feces or urine. If the eggs become trapped in the human host's bladder or liver, however, granulomas (masses of small blood vessels and connective tissue) form around them, and the eggs eventually die and calcify, producing inflammation and scars. The damage done by schistosomiasis is primarily due to the human body's reaction to accumulated eggs and their associated granulomas.

The schistosome eggs that are excreted by the host into freshwater lakes or streams hatch into ciliated larval worms (miracidia). Miracidia then enter an intermediate snail host, in which they proliferate. Immature worms (cercariae) are released from the snail and can rapidly penetrate the unbroken skin of persons who enter infected water (see fig. 4-4). The tailless immature worms (now called shistosomules) enter the human circulatory system and proceed to the liver, where they mature. As they mature, they coat their surfaces with proteins acquired from their human hosts, which enable them to fool the hosts' immune system into tolerating their presence (130). After emerging from the liver, mature worms

Figure 4-4.—Generalized Life Cycle of *Schistosoma*, the Cause of Schistosomiasis



1. Microscopic cercariae are released from snail intermediate host.
2. Cercariae penetrate skin of human host.
3. Tailless cercariae, now called schistosomules, migrate to the human host's small blood vessels, are carried to lungs, then through the heart into the liver to mature; paired (male and female) mature schistosomes lodge in the human host's veins (*S. mansoni* shown).
4. Female schistosomes continually produce eggs (up to thousands each day), which penetrate the human host's intestine (*S. mansoni* and *S. japonicum*) or the urinary bladder (*S. haematobium*), from which they are excreted in feces or urine, respectively.
5. Eggs hatch in fresh water into ciliated miracidia.
6. Miracidia penetrate intermediate snail host, lose cilia, and become sporocysts. Sporocysts reproduce asexually in the snail, proliferating greatly, eventually giving rise to cercariae.

SOURCES: Adapted from T. C. Cheng, *Symbiosis* (New York: Pegasus, 1970) and E. R. Noble and G. A. Noble, *Parasitology*, 3d ed. (Philadelphia: Lea & Febiger, 1973).

mate for life within small veins around either the bowel or bladder area, thus completing the parasite's life cycle.

Incidence

A worldwide survey in 1972 (including 71 countries) estimated that 500 million people were exposed to infection by schistosomiasis and 125 million were infected (169). The various forms of this disease occur in parts of Africa, the Caribbean, South America, and the Orient (see fig. 4-5). In Latin America, recorded schistosomiasis incidence is highest in Surinam, where 385 of every 100,000 inhabitants were infected in 1980 (265). *S. mansoni* is also established in suitable snail hosts in more than half of Brazil, where 10 million people are believed infected, and in parts of Venezuela, where 10,000 more people are thought to have the disease. Foci in the Caribbean occur in the Dominican Republic, Guadeloupe, Martinique, and St. Martin. A few cases have been detected in Montserrat.

Interventions

Over the last 10 years, the incidence and prevalence of schistosomiasis have dropped considerably in several countries. In Japan, the prevalence dropped from 25 percent in 1950 to less than 1 percent in 1973 (169). This formidable decrease was brought about by the concrete lining of irrigation ditches, snail control, land reclamation, environmental sanitation, chemotherapy, and health education. Similar decreases reported for some parts of Egypt, Iran, Puerto Rico, Tunisia, and Venezuela were brought about by more or less the same combination of control methods and improved socioeconomic conditions (169). On the minus side, schistosomiasis has spread to new areas as a result of water impoundment and irrigation projects which create and expand suitable environmental conditions for snail hosts and increase human-snail contact. Areas where large hydroelectric dams are being built, especially in South America, may require special surveillance and assessment.

Chemotherapy against schistosomiasis with the newest generation of chemotherapeutic agents is effective and relatively safe. However, total and complete control in endemic areas is difficult to achieve, since it requires attention to other measures, particularly water supply and sanitation, and treatment of snail breeding sites (molluscisiding).

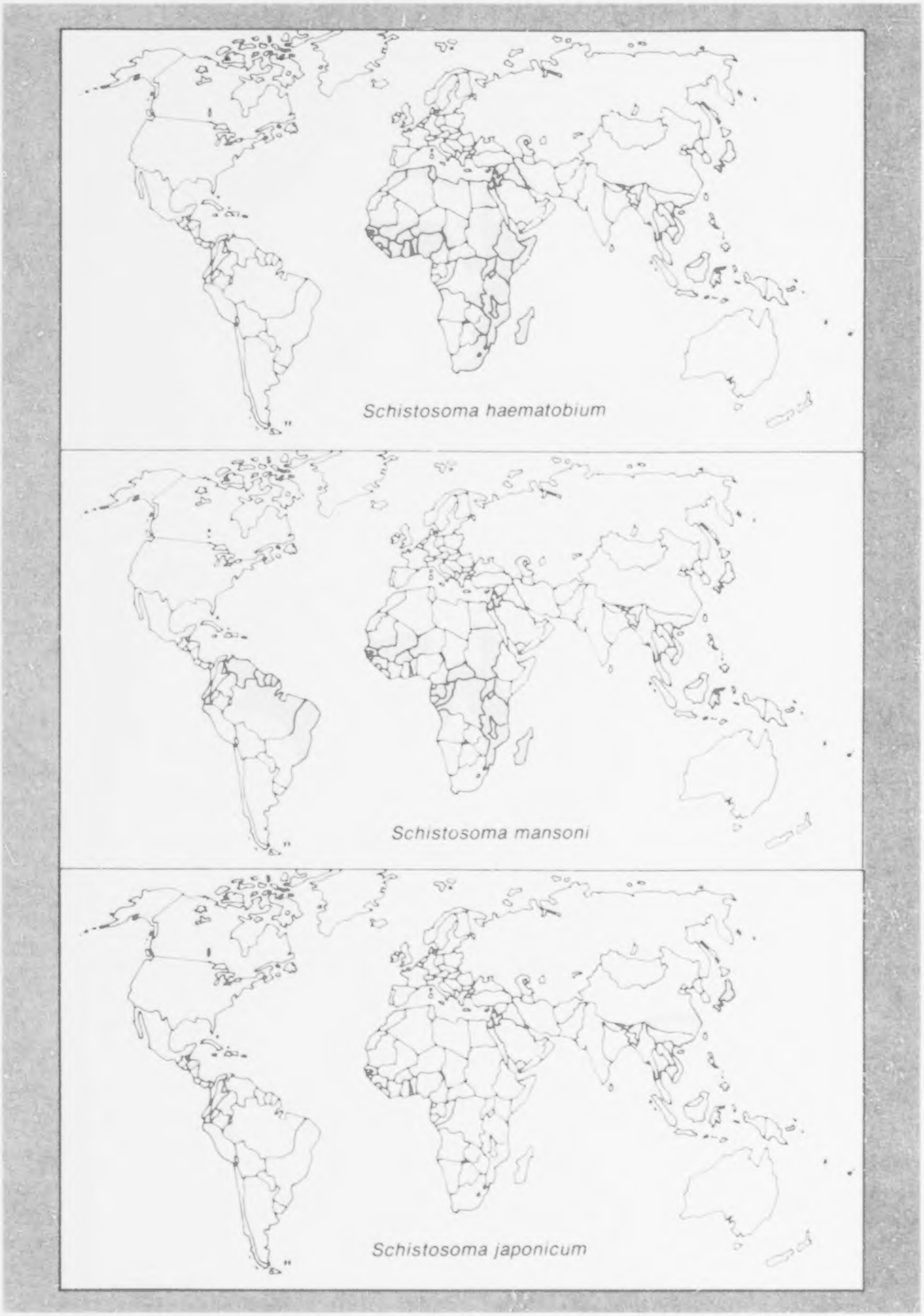
Recent Progress

A fairly large corps of American researchers studies schistosomiasis, and about a dozen laboratories use the techniques of biotechnology. The life cycle of the schistosomiasis parasite is readily adaptable to the laboratory, and small rodents are easily infected. The immunology of schistosomiasis has been studied extensively (39). Most workers are attempting to identify and isolate relevant protective antigens, and many are actively engaged in gene cloning experiments. A live irradiated larval vaccine has been used in cattle in the Sudan with encouraging results (338), but its use in humans is not feasible, because the larvae are alive (though unable to continue their life cycle) when injected.

TRYPANOSOMIASIS

Trypanosomiasis is a general term for two separate diseases caused by protozoan parasites of the genus *Trypanosoma*. *Trypanosoma brucei* infections cause African sleeping sickness (also called African trypanosomiasis), and *T. cruzi* infections cause Chagas' disease (also called American trypanosomiasis). The two diseases have completely different transmission cycles, different vectors, and cause a different pathology. Chagas' disease begins as a blood infection but ultimately attacks various body organs, principally the heart. African sleeping sickness also begins as a blood infection but progresses to central nervous system disease and death.

Figure 4-5.—Geographic Distribution of Schistosomiasis:
Schistosoma haematobium, S. mansoni, and S. japonicum


Schistosoma haematobium
Schistosoma mansoni
Schistosoma japonicum

SOURCES: V. Zaman and L. Keong, *Handbook of Medical Parasitology* (Australia: ADIS Health Science Press, 1982); and H. W. Brown and F. A. Neva, *Basic Clinical Parasitology* (East Norwalk, CT: Appleton-Century-Crofts, 1983), used by permission.

African Sleeping Sickness (African Trypanosomiasis)

Aspects of Natural History

T. brucei, the agent of African sleeping sickness in humans and nagana in livestock, is endemic to large areas of the African continent but does not occur naturally outside of Africa. In the United States, there have been six published reports of imported cases of African sleeping sickness since 1967. All the infected travelers had been on vacation in game parks in eastern or southern Africa, where they were exposed to the tsetse fly vector. The tsetse fly vector of African sleeping sickness is not found in the United States, so there is little danger that the disease will become established in this country (368).

Life Cycle.—There are actually two kinds of African sleeping sickness, each caused by a different variety of *T. brucei* and occurring in its own environmental niche. Both forms of the disease have an early stage involving the blood and lymphatic system and a late stage involving the brain.

In west Africa, sleeping sickness is caused by the parasite *T. brucei gambiense*, which is transmitted by tsetse flies (*Glossina palpalis*) that feed only on humans. The disease is transmitted near streams where tsetse flies breed. *T. b. gambiense* infection usually results in a chronic condition that slowly leads to death.

In east Africa, sleeping sickness is actually a zoonosis (a disease of animals that can be transmitted to humans) caused by *T. b. rhodesiense* and transmitted by a different species of tsetse fly (*G. morsitans*). Humans acquire the disease when they venture into areas, such as hunting grounds and grazing lands, where animals are infected. The disease is fatal to humans within weeks or months and very destructive to livestock. Large expanses of land have become totally unusable because of the disease risk to humans entering them.

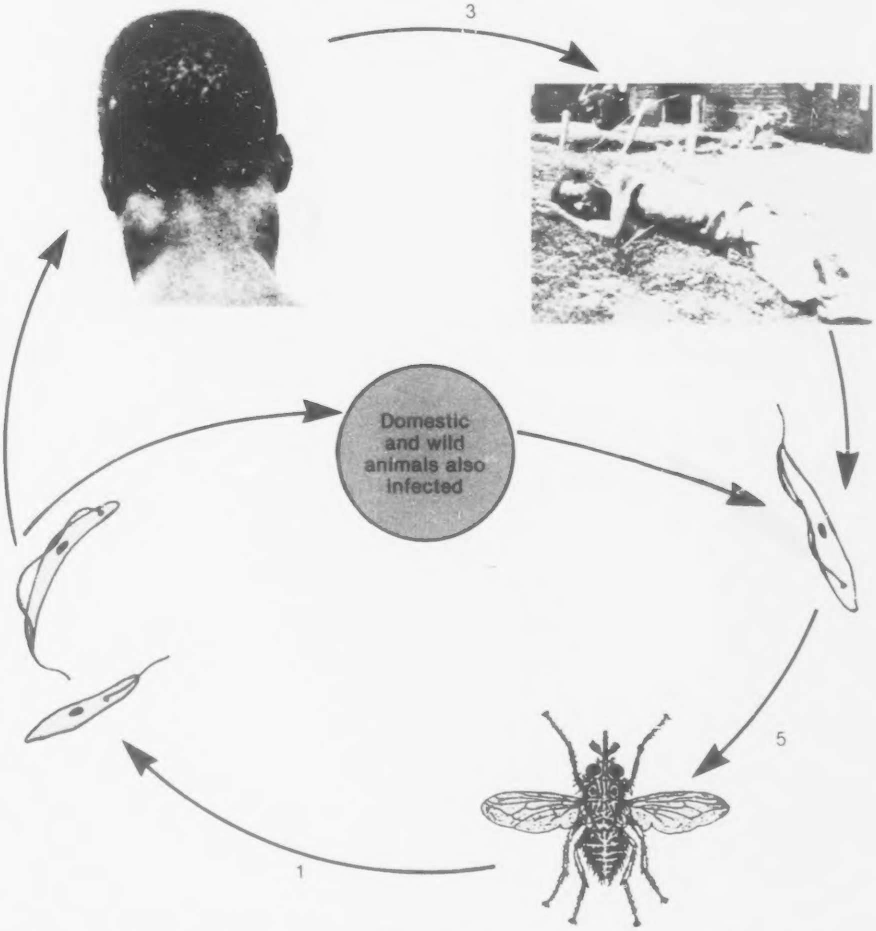
Either form of African sleeping sickness begins when an infected tsetse fly feeds on a human or animal host, depositing trypanosomes in the wound (see fig. 4-6). The trypanosomes invade the host's blood, lymph, and tissue fluids. In cyclical waves, the trypanosomes wane, then reappear, as the body's immune system and the parasites interact. These parasites can undergo frequent changes in a specific surface protein, known as variant surface glycoprotein (VSG) or variant surface antigen. Located on the blood form of the trypanosome, VSGs stimulate the production by the host of a particular responsive and protective antibody.

Within any individual human or animal host, the VSGs of the trypanosome population shift over time through a repertoire of changes numbering potentially over 100, each having no exposed determinants in common with other past or future populations. Two distinct mechanisms appear to be responsible for the VSG changes, which are called antigenic switching. Each mechanism generates cohorts of parasites to which the mammalian host has not yet made antibody. Since antibodies raised against one variant are generally not effective against other variants, the trypanosome is able to evade the host's immune response.

Until the host can mount another antibody response, symptoms of fever, headache, and joint pain occur. As the infection progresses, lesions develop in the brain, heart, and small blood vessels. Ultimately, nervous system involvement develops, first with signs of insomnia and excitability, then coma and death. Symptoms and outcome are similar in both the acute and chronic forms, but the duration differs.

Incidence.—Recent estimates put 45 million people at risk of sleeping sickness infection in Africa. Until 1979, about 10,000 new cases were recorded annually, but since then, serious outbreaks have resulted in more than 20,000 new cases recorded per year (353). Because of the difficulties of diagnosing chronic cases and the usual occurrence of the disease in rural areas, these figures are probably underestimates. Although widespread across Africa (see fig. 4-7), sleeping sicknesss occurs in well-defined endemic foci because the tsetse fly vectors breed in rivers and streams. Unfortunately, those same water sources are essential to humans and grazing animals.

Figure 4-6.—Generalized Life Cycle of *Trypanosoma brucei gambiense* and *T. b. rhodesiense*, the Causes of African Sleeping Sickness (African Trypanosomiasis)



1. Trypanosomes develop in tsetse fly vector to a form infective to humans and other mammals.
2. Humans and animals are infected through bite of tsetse fly. Enlarged lymph nodes are an early sign of trypanosome infection.
3. Trypanosomes eventually invade the human host's central nervous system, causing classical symptoms of sleeping sickness, leading to coma and death.
4. Forms of trypanosome infective to tsetse fly vector are released into blood circulation.
5. Tsetse fly picks up infective form of trypanosome while biting infected human or other infected mammal.

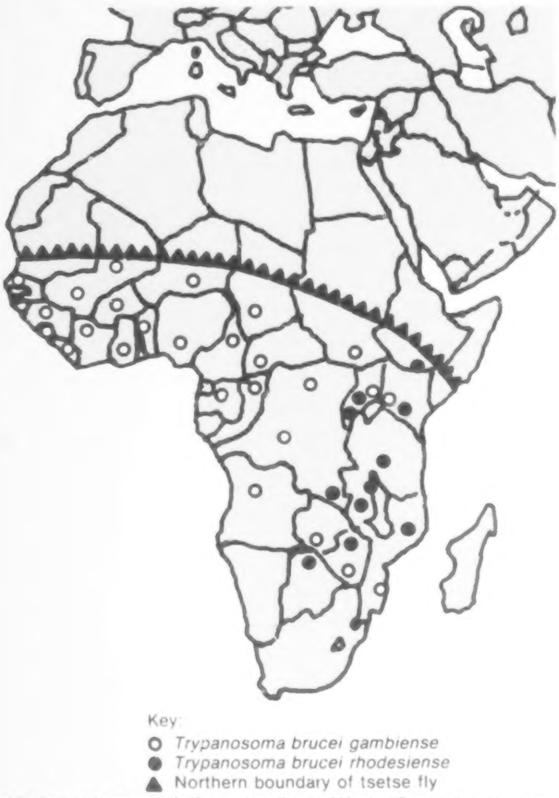
SOURCE: Office of Technology Assessment, 1985. Photos from H. W. Brown and F. A. Neva, *Basic Clinical Parasitology* (East Norwalk, CT: Appleton-Century-Crofts, 1983), used by permission.

Interventions

Treatment for African sleeping sickness requires hospitalization because of the dangerous side effects and toxicity of the drugs, as well as the need for their intravenous administration. Although the infection is uniformly fatal without treatment, there is a risk (2 to 5 percent) of succumbing to the treatment itself.

Control of the tsetse fly vectors of sleeping sickness is currently the only feasible means of intervention. Because of the focal nature of transmission, insecticiding is feasible (no insecticide

Figure 4-7.—Geographic Distribution of African Sleeping Sickness (African Trypanosomiasis)


Key:
○ *Trypanosoma brucei gambiense*
● *Trypanosoma brucei rhodesiense*
▲ Northern boundary of tsetse fly

SOURCE: V. Zaman and L. Keong, *Handbook of Medical Parasitology* (Australia: ADIS Health Science Press, 1982), used by permission.

resistance exists, as yet, in the vectors) and has been used successfully, though it is expensive, contaminates the environment, and is labor-intensive. In some cases, clearing vegetation near river breeding sites has worked. Overall, vector control has met with only limited, sporadic success. Recent efforts have focused on the use of insecticide-impregnated traps that attract the vector flies.

Recent Progress

Since about 1979, trypanosomes have become increasingly popular for studies in molecular biology. The organisms of the *T. brucei* complex are becoming the *Drosophila* (fruit flies) of modern molecular biologists. The purpose of most of the studies using these trypanosomes has been to work out the intricacies of gene coding, transcription, translation, and expression, rather than to control African sleeping sickness. These studies have made many contributions to the fundamental understanding of gene function, however, and there is hope that useful spinoffs can be applied to controlling sleeping sickness.

More disease-focused work by American investigators is centered largely on the molecular biology of antigen switching of VSGs. Monoclonal antibodies (MAbs) to different VSGs have been produced in several laboratories (43). One laboratory deals exclusively with the nonvariant antigens and regulatory proteins, studying the stage-specific expression of membrane and internal proteins and host reactions to them. In this laboratory, an attempt is being made to find proteins that could be used in immunodiagnosis and metabolic targets for chemotherapy (16).

Chagas' Disease (American Trypanosomiasis)

Aspects of Natural History

Chagas' disease is caused by the protozoan parasite *T. cruzi*. Though sometimes congenital and occasionally transmitted through blood transfusion (308), Chagas' disease is primarily transmitted to humans (and other mammals) by reduviid bugs, blood-sucking insects found throughout Central and South America. Thus, it is primarily a disease of poor rural areas, where adobe brick houses and thatch roofs provide harboring sites for reduviid bugs to live and breed.

Several strains of *T. cruzi* exist in different parts of Latin America. The strains vary considerably in the pathology they produce in humans because of the range of immune system reactions they can evoke. About 150 species of mammals, including dogs, cats, guinea pigs, opossums, rats, and other rodents, are thought to be reservoir hosts of *T. cruzi*.

There is no effective cure for Chagas' disease. In the acute phase of the disease, when the parasites are invading internal organs, headaches, fever, anemia, and exhaustion may occur. The severity of this phase varies with the age of the patient. The younger the patient, the more severe the disease. Thus, children under the age of 2 may die, while adults may exhibit no symptoms. The

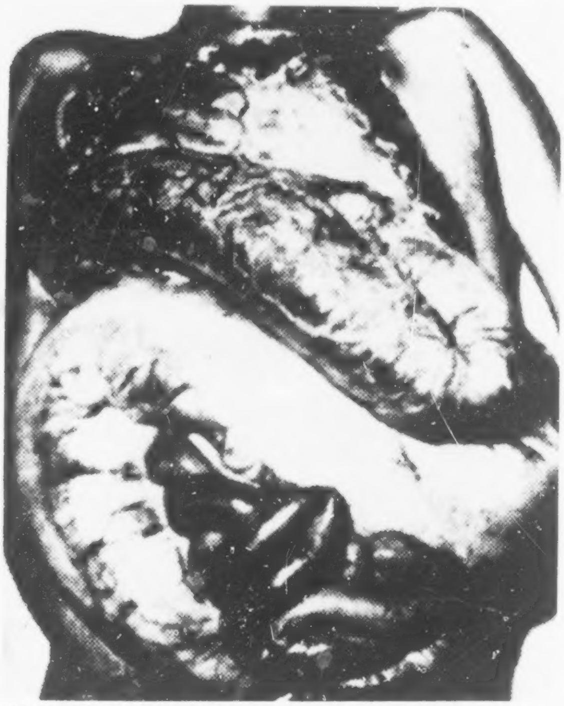


Photo credit: H. W. Brown and F. A. Neva, "Basic Clinical Parasitology," Appleton-Century-Crofts, 1983. Reprinted by permission.

Megacolon, one effect of Chagas' disease.

acute stage of Chagas' disease may resolve completely in a few weeks or months or instead may pass into a subacute or chronic stage.

Long-term sequelae of Chagas' disease include grotesque enlargement of the digestive tract (megaesophagus and megacolon), circulatory problems, and central nervous system damages and most seriously, damage to the heart muscle, sometimes leading to death from heart failure.

Life Cycle.—T. cruzi infection in humans results when an infected reduviid bug bites a person, usually around the eye while the person is sleeping, and deposits feces containing *T. cruzi* parasites into the bite wound (see fig. 4-8). *T. cruzi* has two life stages in the mammalian host, one that circulates in blood and another that proliferates intracellularly within the tissues. Forms of the parasites infective to reduviid bugs are released into the mammalian host's circulation, ready to be picked up by reduviid bugs in the course of another insect bite.

Incidence.—Chagas' disease occurs in almost every country of Latin America: Brazil, Peru, Venezuela, Chile, Bolivia, Paraguay, Uruguay, Argentina, Colombia, Mexico, Costa Rica, and Panama (see fig. 4-9) (265). Although the vectors and reservoir hosts are also present across the Southern United States, only three indigenous cases of Chagas' disease have been reported. Two were infants reported to have contracted the disease in Texas in 1955, and the third was a woman from the Sacramento Valley area in California in 1982. None of the three had previously had contact with pets carrying the disease or had recent blood transfusions or had been outside the country (309).

It has been estimated that about 12 million of the 50 million exposed people living in endemic areas are infected with *T. cruzi* (229). Since notification of authorities regarding the presence of Chagas' disease is not compulsory, there are no reliable morbidity data. Studies in Brazil have shown Chagas' disease to be a significant cause of mortality in people under 45 years of age (287) and a heavy burden to society because of the need for hospitalization and disability assistance (268).

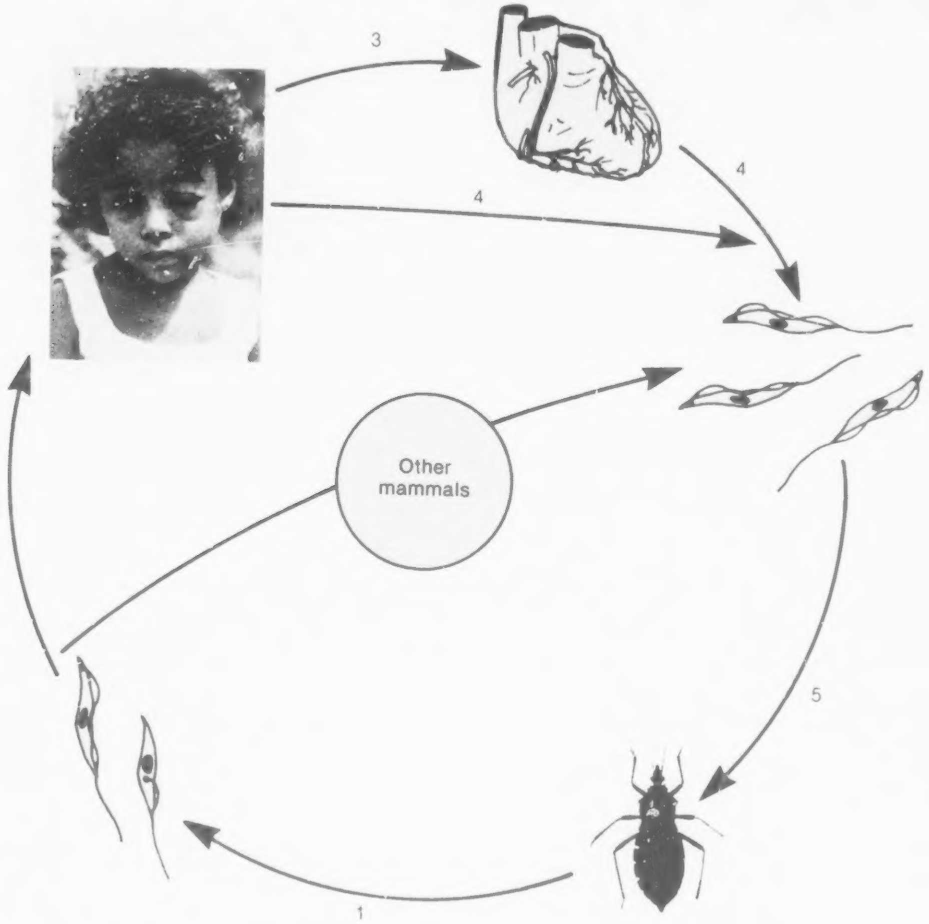
Interventions

Control measures for Chagas' disease concentrate on insecticide spraying of houses and upgrading of housing construction. In one area of Venezuela, the use of insecticides was believed to account for a significant decline in the percentage of the population infected with *T. cruzi* during the 1970s (29). Vector bionomics remains an important research topic for defining transmission areas, vector behavioral characteristics, and improved control measures.

Recent Progress

In an attempt to understand why the human host's immune system is not effective in controlling Chagas' disease, some investigators have examined the proteins produced by *T. cruzi*. There is no evidence for VSGs in *T. cruzi* (322), although a large number of local genetic strains do exist. Some other mechanism must be responsible for *T. cruzi*'s ability to evade the human immune system.

Figure 4-8.—Life Cycle of *Trypanosoma cruzi*, the Cause of Chagas' Disease

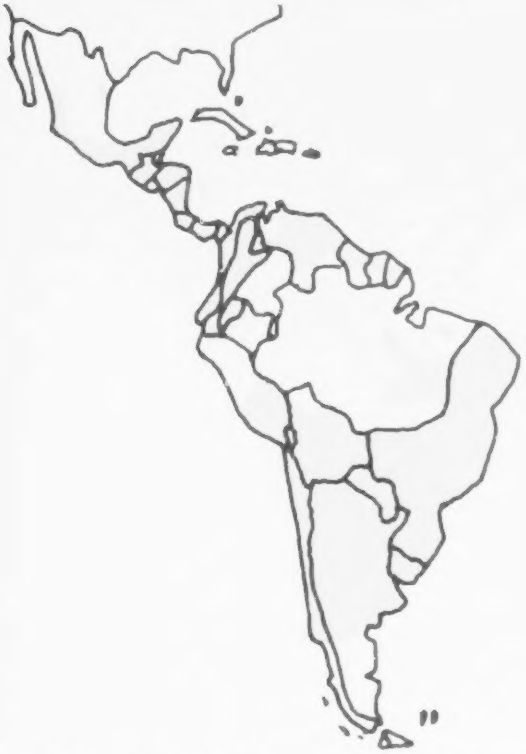


1. Trypanosomes develop in the reduviid bug vector to a form infective to humans and other mammals.
2. Parasites deposited on human skin in insect feces at time of insect bite invade human through the bite wound. "Romana's sign," the swelling of one eye, is a sign of a reduviid bite.
3. Trypanosomes enter and multiply in muscle tissue, including the heart.
4. Forms of trypanosomes infective to reduviid bug vector are released into blood circulation.
5. Reduviid bug picks up infective form of trypanosome while biting infected human or other infected mammal.

SOURCE: Office of Technology Assessment, 1985

Major surface proteins isolated from *T. cruzi* organisms resembling the insect stages of the parasite (grown in culture) and from mammalian blood stages were recognized by immune sera from naturally infected humans and experimentally infected mice (259). This means the immune system raises antibodies against both insect and blood stages of the parasite. MAbs to culture and intracellular stages of *T. cruzi* have been made by several workers, and Snary and colleagues (323) have demonstrated that the antigenic determinants are strain-specific. The ideal vaccine must

Figure 4-9.—Geographic Distribution of Chagas' Disease (American Trypanosomiasis)



SOURCES: World Health Organization, Geneva, Switzerland, and London School of Hygiene and Tropical Medicine.

produce antibodies that do not cross-react with heart and nerve tissue and that are effective against all strains and stages of *T. cruzi*.

The development of an effective therapeutic drug for Chagas' disease is a critical research need. With a therapeutic drug in hand, a simple effective test for early diagnosis of the disease would be essential, for once the long-term effects appear they are irreversible. Vaccine research is under way, but because the long-term pathology seems to result from the body's immune response against the parasite and cross-reacting with its own heart and nerve tissue, prospects for an effective vaccine are uncertain.

Fewer than 10 laboratories in the United States focus on *T. cruzi*. Most are in academic institutions, with one or two each in government and in industry. Significant research is taking place in South America, as well as in a few European laboratories. In part because of this dearth of effort, work on Chagas' disease has not advanced as much as that on some other diseases (16).

LEISHMANIASIS

Aspects of Natural History

Leishmaniasis is the collective term for the diseases caused by several species of the protozoal genus *Leishmania*. Among the protozoan diseases, leishmaniasis is commonly considered second in importance, following malaria.

Depending on the infecting species, leishmaniasis may appear in different forms. Cutaneous leishmaniasis, which appears as self-limiting and usually self-resolving sores located at the point of infection, is caused by *L. mexicana*, *L. braziliensis*, or *L. tropica*. Mucocutaneous leishmaniasis, which also begins as a sore, is caused by certain geographic strains of *L. braziliensis* that commonly metastasize and proliferate in the nasal and pharyngeal mucous membranes. Gross destructive disfigurement of the face, nose, and throat results.

A third type of this leishmaniasis is a visceral form, caused by *L. donovani*. In this form, called "kala azar," the spleen, liver, bone marrow, and lymph glands are the sites of parasite proliferation. Fatal outcome in children is common. Kala azar occurs sporadically in many tropical areas, but it has also appeared in epidemics, killing many thousands of people at a time in southern Asia. Recent epidemics of kala azar have resulted from the reemergence of the sandfly vector after spray programs were discontinued.

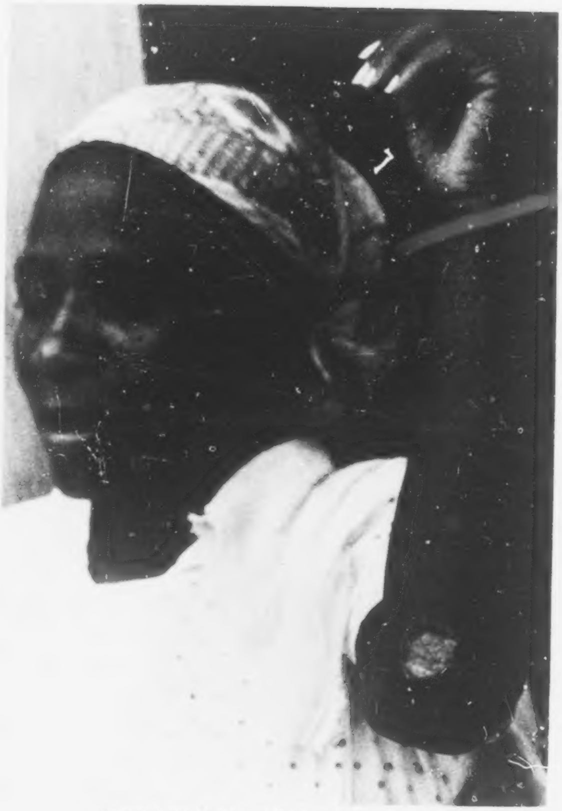


Photo credit: Dr. Robert Edelman, National Institutes of Health

A lesion of cutaneous leishmaniasis.

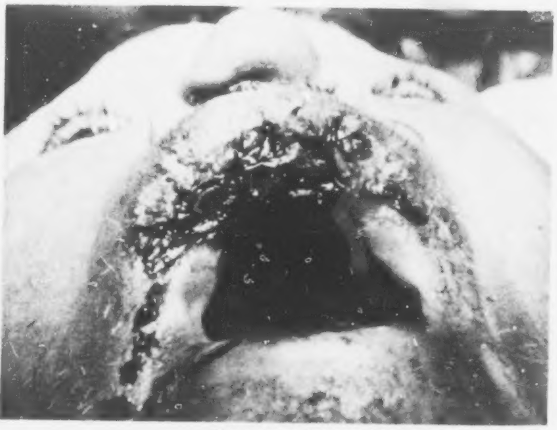


Photo credit: Office of Technology Assessment

Destruction of tissue resulting from mucocutaneous leishmaniasis.

Life Cycle

All types of leishmanial parasites are transmitted by blood-sucking phlebotomine sandflies, in which the parasitic organism undergoes part of its complex life cycle (see fig. 4-10).

Leishmania invade and multiply within the host's macrophages, cells that are part of the immune system. Since macrophages are specialized for ingestion and destruction of most foreign organisms, the ability of leishmanial organisms to live in them is paradoxical. A key to controlling leishmaniasis may be to identify a mechanism that will activate macrophages to kill *Leishmania* and then to attempt to activate this mechanism with a vaccine or drug.

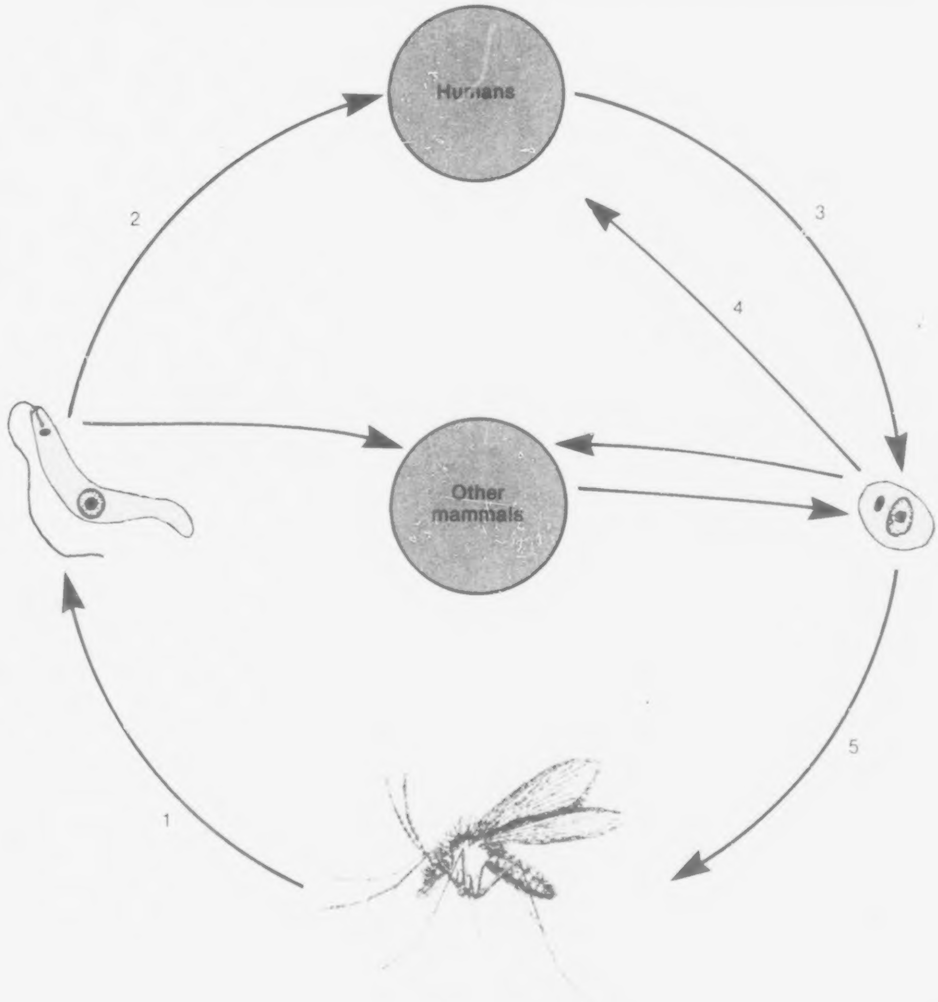
Incidence

The various forms of leishmaniasis are distributed widely, along the U.S. Texas/Mexico border, in Latin America, in the Mediterranean, and in Africa, India, and China (see fig. 4-11). Incidence rates range from a low in Guatemala of 1.2 per 100,000 in 1980, to a high of 60.5 per 100,000 in Costa Rica for the same year. A total of about 400,000 new cases were reported worldwide in 1977, 100,000 of which were in Bihar, a province of India where a severe epidemic of kala azar was occurring (341).

In some countries, the number of cases of leishmaniasis is increasing because of agricultural colonization of jungle areas. Most forms of the disease are transmitted to humans (via sandflies) from animals native to the jungle where the disease occurs. This makes living or working in areas in or near jungles a major health hazard. In the late 1970s, cutaneous leishmaniasis seriously impeded a Bolivian scheme to relocate people outside the overcrowded *altiplano*. Many of the colonists abandoned their land. More than 60 percent of the people who did said that leishmanial disease was their reason for returning to the mountains. Oil exploration and roadbuilding in several Andean countries have also been significantly hampered (265).

A seroepidemiologic survey in Panama revealed an apparent focus of leishmanial transmission, without detecting the presence of clinical infec-

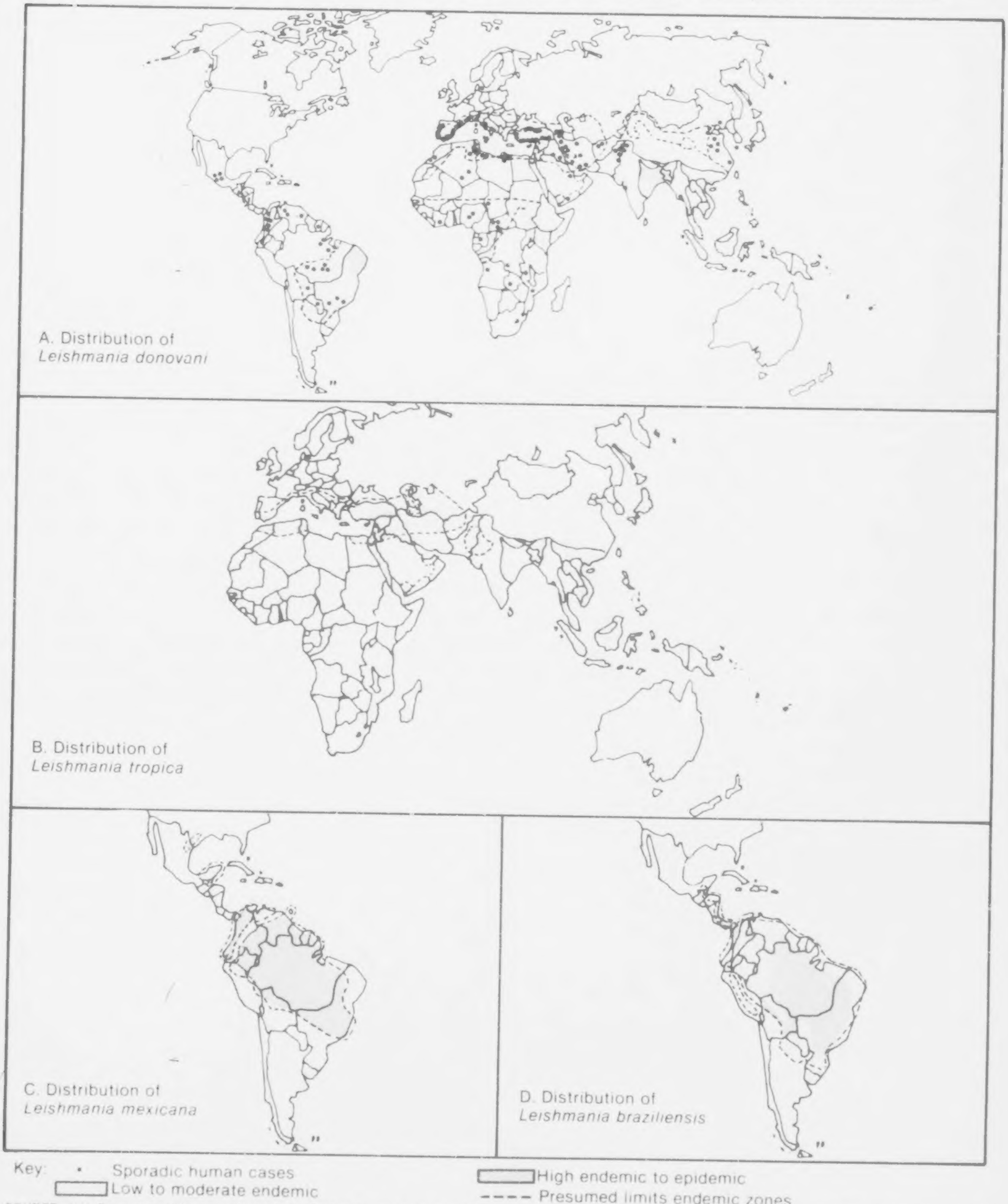
Figure 4-10.—Generalized Life Cycle of *Leishmania*, the Cause of Leishmaniasis



1. Parasites develop to "promastigote" stage infective to humans and other mammals in sandfly vector.
2. Parasites are transmitted to humans and other mammals through bite of sandfly.
3. Parasites transform to "amastigote" stage in mammalian host, multiplying within certain cells of the host's immune system.
4. Some amastigotes infect new cells in the mammalian host.
5. Some amastigotes are picked up by sandflies during bite of infected mammalian host.

SOURCE: Office of Technology Assessment, 1985. Photo from H. W. Brown and F. A. Neva, *Basic Clinical Parasitology* (East Norwalk, CT: Appleton-Century-Crofts, 1983), used by permission.

Figure 4-11.—Geographic Distribution of Leishmaniasis Caused by Four Species of Leishmania



A. Distribution of *Leishmania donovani*

B. Distribution of *Leishmania tropica*

C. Distribution of *Leishmania mexicana*

D. Distribution of *Leishmania braziliensis*

Key:
• Sporadic human cases
▭ Low to moderate endemic
▭ High endemic to epidemic
---- Presumed limits endemic zones

SOURCE: U.N. Development Program/World Bank/WHO, Special Program for Research and Training in Tropical Diseases, *Sixth Programme Report: 1 July 1981—31 December 1982* (Geneva: WHO, 1983).

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tion. Completely subclinical leishmaniasis was previously unknown and may be an important clue to vaccine development.

Interventions

For disease caused by most species of *Leishmania*, no effective prevention is known. However, immunity to Old World cutaneous leishmaniasis (also called Oriental sore) arising from *L. tropica* infection can be induced by inoculation of a susceptible person with organisms from an active lesion, a procedure apparently known from ancient times in endemic regions.

Specific treatment for leishmaniasis is now limited to antimony compounds. These compounds are not always effective and often have adverse toxic side effects. Another disadvantage of these compounds is that they require daily injections for 10 to 20 days, making them impractical for patients living in remote and inaccessible areas. Hospitalization for such a period is not only expensive but also a major inconvenience to patients who cannot afford to leave work or their farms for an extended period. For these reasons, improved treatment of the tens of thousands of existing cases is a priority research goal. The Pan American Health Organization/WHO is attempting to foster development of new therapeutic drugs. One, allopurinol, in combination with other drugs, is a promising new treatment (393).

Recent Progress

Fewer than 10 laboratories in the United States study leishmanial organisms. The difficulty in distinguishing between *Leishmania* species (necessary to properly predict clinical outcomes and select treatment) has been a persistent problem. The solution, however, may be aided by biotechnology. In recent years, MAbs have been prepared against a variety of antigenic determinants in *Leishmania* spp. (16) and used to probe morphologic and taxonomic differences. Rapid identification of *Leishmania* spp. may soon be possible with a recently published technique of DNA hybridization (405).

A variation of the enzyme-linked immunosorbent assay (ELISA), called the "DOT-ELISA" method (267), is also a major advance, allowing for rapid easy field diagnosis. These and other methods would permit early treatment of the destructive mucocutaneous form of the disease and would also facilitate precise epidemiologic field studies.

The possibility of developing a vaccine against the promastigote form of the leishmaniasis parasite (the stage transferred through the bite of the sandfly) remains. Such a vaccine would have limited usefulness because it would be ineffective against the amastigote form of the parasite (the stage which lives within the macrophage). The transformation from promastigote to amastigote occurs rapidly within the host, before a vaccine-initiated antibody response could be effective.

FILARIASIS

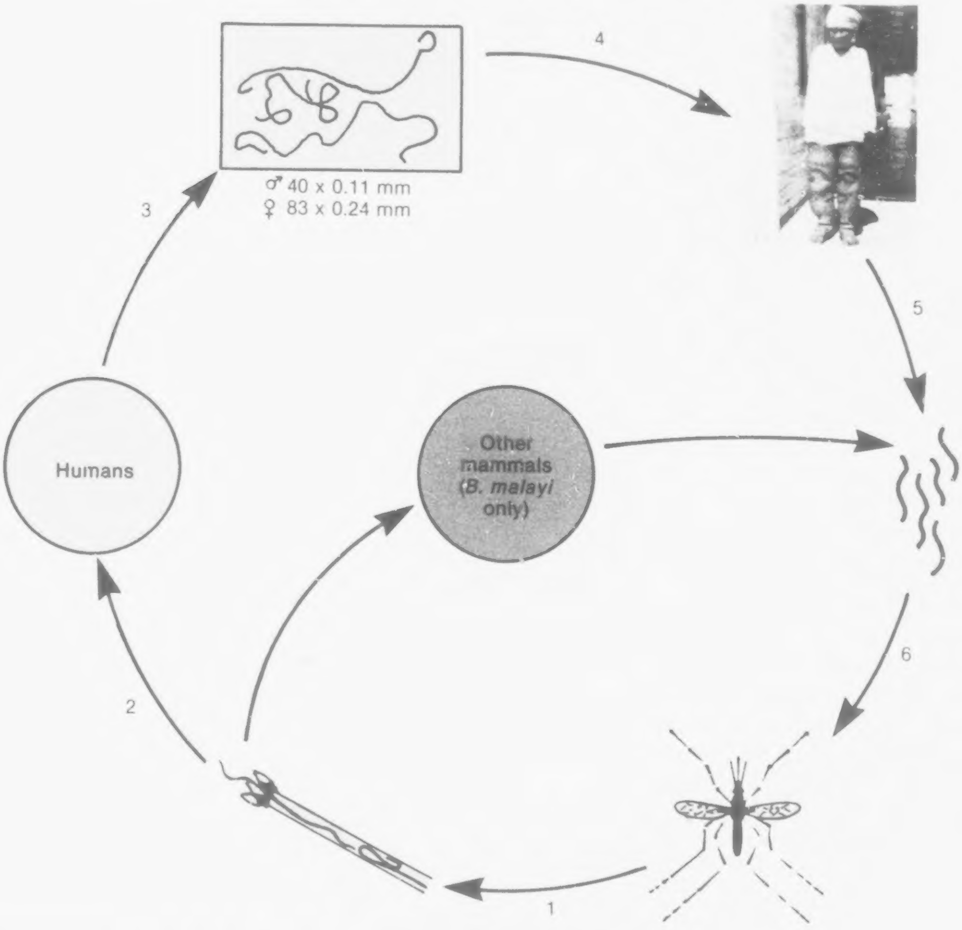
Aspects of Natural History

Filariasis is a collective term for several distinct parasitic infections of tissue-dwelling, threadlike nematodes, which are transmitted by mosquitoes and other insects. There are at least eight different types of human filarial infections, among them infection by the infamous guinea worm, which before modern chemotherapy evolved was removed by gradually winding the protruding worm around a stick. Probably the most important of the filarial parasites, in terms of worldwide prevalence and severity of disease, are *Wuchereria bancrofti* and *Brugia malayi*, which cause lymphatic forms of filariasis; and *Onchocerca volvulus*, the agent of onchocerciasis (river blindness).

Life Cycles and Interventions

The life cycles of *W. bancrofti* and *B. malayi* are fairly similar (see fig. 4-12). These organisms are transmitted to humans by several species of mosquitoes, including common household pest species. The adult filarial worms live in the human host's lymphatic system and cause pathol-

Figure 4-12.—Generalized Life Cycle of Two Important Filarial Worms: *Wuchereria bancrofti* and *Brugia malayi*



1. Infective larvae develop, but do not multiply, in mosquito vector.
2. Larvae deposited on skin of human or other mammalian host (*B. malayi* only) through mosquito proboscis at time of bite.
3. Larvae penetrate skin, migrate to host's lymphatic system, and mature.
4. Masses of adult worms can block lymph vessels, causing accumulation of lymph fluid and growth of lymph tissue, in a manifestation called elephantiasis.
5. Adult worms release immature microfilariae into blood circulation, usually in a circadian rhythm.
6. Mosquitoes ingest microfilariae during bite of infected human or other mammal.

SOURCE: Office of Technology Assessment, 1985. Photo from F. W. Brown and F. A. Neva, *Basic Clinical Parasitology* (East Norwalk, CT: Appleton-Century-Crofts, 1983), used by permission.

ogy that differs with the host's immune response. Inflammation and gross obstruction results in varying degrees of swelling of the lymph glands, which may result grotesque enlargement (elephantiasis) of the legs, breasts, or scrotum. Adult worms release immature forms (microfilariae) that circulate in the human blood and then infect feeding mosquitoes to complete the transmission cycle. Drugs are available to kill the microfilariae, but the means to kill mature worms are poor.

O. volvulus parasites are transmitted to humans by blackflies of the genus *Simulium*. These blackflies require running water to complete their life cycles, limiting their habitat mainly to areas around rivers (hence the name river blindness for onchocerciasis). The adult parasitic worms live in the tissues of the human body and often form large nodules where an intertwined clump of worms localizes. Microfilariae released by the adult worms migrate through the human host's body in subcutaneous tissues where they can be picked up by feeding blackflies. When microfilariae reach the human eye, blindness can result. The prevention of blindness is imperfectly achieved by chemotherapy and surgical removal of the nodules. Few preventive measures are available, and larvicides used to control the blackfly vector of onchocerciasis are subject to resistance and have only transient effects.

Incidence and Prevalence

Filariasis due to *W. bancrofti* has a wide but focal urban distribution throughout the Pacific region, Asia, Africa, and Latin America (see fig. 4-13) (406). Lymphatic filariasis caused by *B. malayi* is primarily found in rural foci in Sri Lanka, Thailand, Malaysia, Vietnam, China, South Korea, Borneo, and Indonesia. Onchocerciasis is found in central and western Africa, North Yemen, Saudi Arabia, Mexico, Venezuela, Colombia, Brazil, Ecuador, and Central America.

It is estimated that bancroftian filariasis and onchocerciasis are more prevalent today than they were more than 100 years ago (256). More than 300 million people are exposed to mosquito-transmitted lymphatic filariasis, and more than 30 million are infected. The main endemic areas in India remain, and there is little control of the disease in Africa, where in savannah areas it is estimated that more than 15 percent of the adults are infected.

Obstacles to Research

Considering the great number of people affected or at risk, their widespread geographical distribution, and the severity of their pathology, the filarial diseases are relatively neglected by American researchers. A major obstacle to research on filariasis is the difficulty in maintaining filarial organisms for laboratory study. Their complicated life cycles and the unavailability of suitable animal models make these parasites among the most frustrating to work with.

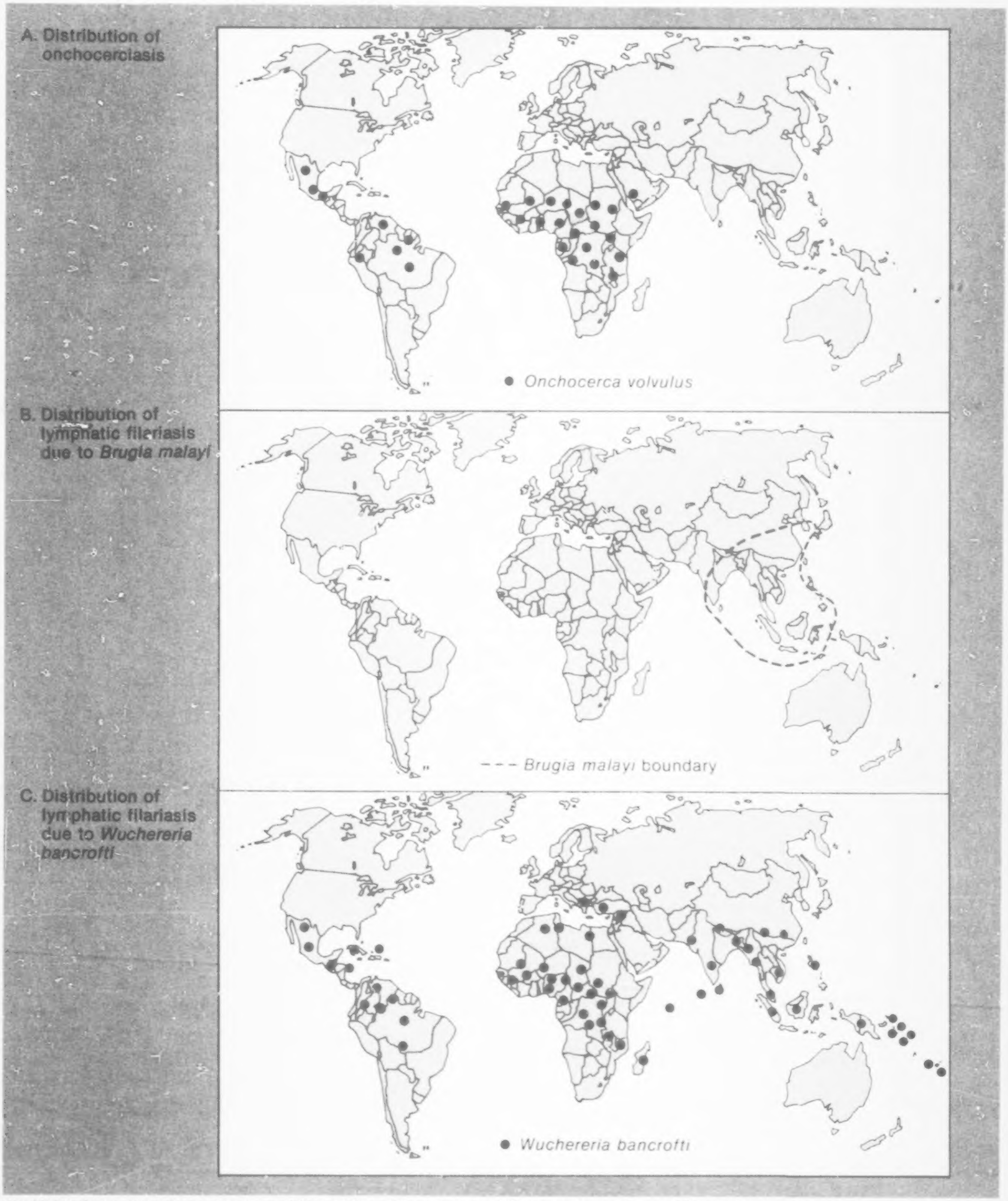
LEPROSY (HANSEN'S DISEASE)

Aspects of Natural History

Leprosy is a chronic bacterial infection that continues to be an important public health problem in many countries. The disease is caused by *Mycobacterium leprae*, a bacterium similar to the one that causes tuberculosis. Leprosy is mainly a disease of the skin and peripheral nerves, but it is characterized by a wide array of clinical presentations.

Much of the pathology of leprosy is associated with a defective cell-mediated immune response in certain individuals. Depending on the host's immunologic response, leprosy ranges from benign tuberculoid leprosy, with localized skin lesions and nerve involvement (sometimes severe peripheral neuropathy) and the presence of few *M. leprae* bacteria, to lepromatous leprosy, with spreading lesions that become nodular and dis-

Figure 4-13.—Geographic Distribution of Major Filarial Diseases: Infection With *Onchocerca volvulus*, *Brugia malayi*, and *Wuchereria bancrofti*



A. Distribution of onchocerciasis

B. Distribution of lymphatic filariasis due to *Brugia malayi*

C. Distribution of lymphatic filariasis due to *Wuchereria bancrofti*

● Onchocerca volvulus
--- Brugia malayi boundary
● Wuchereria bancrofti

SOURCE: V. Zaman and L. Keong, *Handbook of Medical Parasitology* (Australia: ADIS Health Science Press, 1982), used by permission.

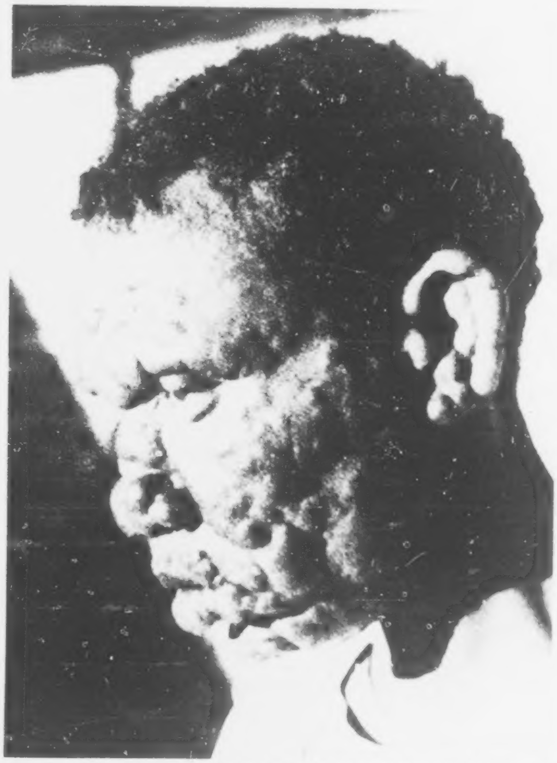


Photo credit: Office of Technology Assessment

Lepromatous leprosy patient.

figuring, resulting in destruction of the nose, involvement of the vocal cords and eyes, and often severe nerve damage, with a heavy infection of *M. leprae*.

Questions about why leprosy has such varied effects on its victims have not been fully answered. Researchers are not even sure how the disease is transmitted. The latent period, the time between infection and the actual appearance of symptoms, often lasts for many years. The individual is capable of unknowingly infecting others during this time. Leprosy is more likely to be spread by chronic exposure to dried skin lesion matter and nasal secretions from the lepromatous and more severe borderline patients. The general consensus seems to be that *M. leprae* bacteria enter the human body through the respiratory system, although some researchers suspect entry may also be through the skin (406).

Immunologic diagnostic techniques have shown that people with tuberculoid leprosy have a strong and effective cell-mediated immune response (via lymphocytes) that controls the infection, whereas lepromatous leprosy patients do not. There is some evidence to suggest a genetic basis for this difference. The nerve damage in patients with lepromatous leprosy seems to result from an absence of cellular immune response. Characterization of this defect in lepromatous leprosy patients is being investigated with MAbs that can identify lymphocyte subsets and also by analysis of the patients' genetic type.

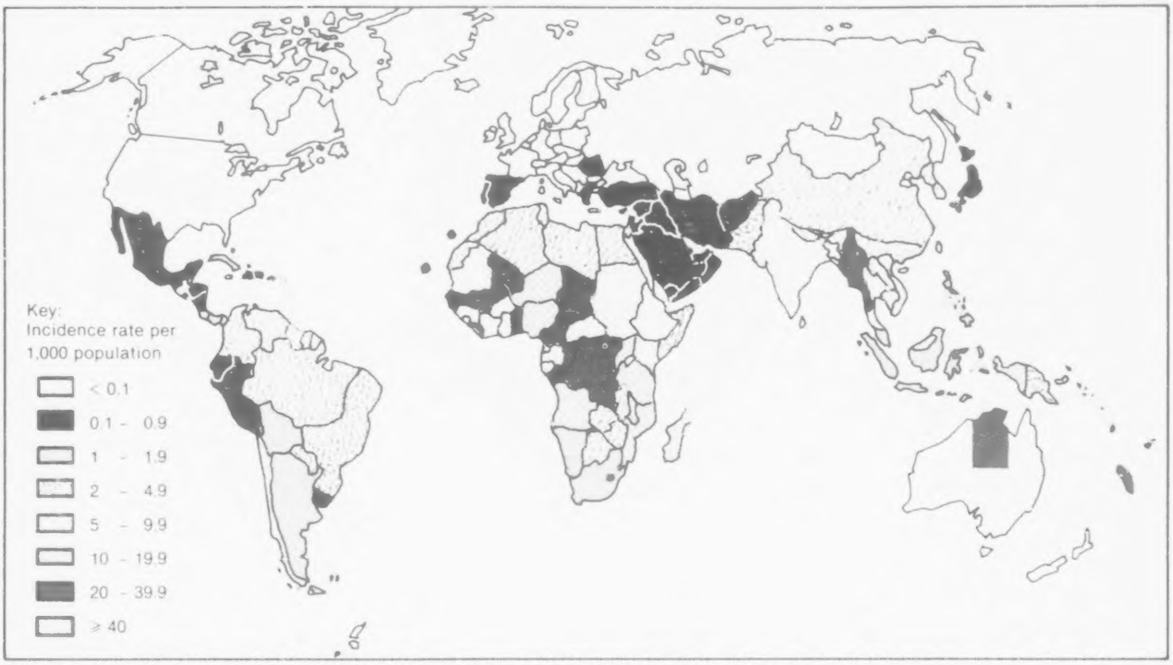
Incidence and Prevalence

Worldwide there are an estimated 15 million leprosy cases (307). The prevalence of leprosy has been reduced in many places, but the overall incidence (i.e., the number of new cases per year) has not changed with advances in science (see fig. 4-14). In some very small, isolated communities in parts of Africa and Australia, the prevalence of leprosy may be as high as 1 out of every 50 inhabitants. The disease is also common in southern Asia, especially India (210). In China, an estimated 500,000 cases of leprosy occurred in the early 1950s, but fewer than 200,000 cases were reported in 1984 (307). As of 1984, there were 2.5 million cases in Southeast Asia alone (135).

In the Americas, there are about 400,000 cases of leprosy, 80 percent of the new cases occurring in Argentina, Colombia, and the Amazon area of Brazil (265). The recorded incidence of leprosy in the Americas has almost doubled in the last 10 years, but the increase is thought to reflect improvements in case finding and notification of authorities, rather than to be a true change. Over half the clinical cases are of the more severe lepromatous form. However, as many as four-fifths of those infected with *M. leprae* never get sick, though they may transmit the disease to those with more susceptible immune systems (210).

About 20 cases of leprosy acquired in the United States are diagnosed each year, occurring in Texas, Louisiana, California, Florida, and Hawaii. Of the 250 new cases of leprosy diagnosed in the United States in 1982, 233 were immigrants

Figure 4-14.—Geographic Distribution of Leprosy



SOURCE: L. M. Bechelli and V. Martinez Dominguez, "The Leprosy Problem in the World," *Bull. W.H.O.* 34:811-826, 1966.

who had contracted the disease in their homelands. In the past, most cases have come from Mexico and the Philippines, but in recent years immigrants from Cuba, Haiti, El Salvador, Nicaragua, and Southeast Asia have entered the United States with leprosy (135).

Why leprosy occurs more in some parts of the world than in others is not fully understood.

Interventions

A number of useful drugs are now available for leprosy treatment. The most effective drug is dapsone. Unfortunately, however, strains of *M. leprae* have developed resistance to dapsone (217). This situation has resulted in new recommendations for combination chemotherapy for leprosy that will shorten the treatment period and increase the likelihood of effective control of the disease. If organized and administered well, combination chemotherapy will lighten the workload of health services, improve patient compliance, and result in better prognosis.

Vaccination against leprosy is in the human trial stage, but still years from proof of its effectiveness and routine use. Research on the epidemiology of leprosy is still needed to improve intervention strategies.

Recent Progress

A small number of laboratories in the United States, probably fewer than 10 (including the U.S. Public Health Service in Carville, LA) specialize in leprosy research. Past leprosy research efforts were hampered by the inability of researchers to grow *M. leprae* in culture and also by the lack of a suitable animal model. The introduction of the armadillo model of infection in the early 1970s has helped both in the characterization of the disease and in the development of serodiagnostic procedures and a potential vaccine (307). The fairly recent discovery of the susceptibility of mangabey monkeys (217) may also help research efforts. The problems at this point are the slow growth rate of *M. leprae* in culture and the inability of armadillos to breed in captivity, necessitating their continual trapping from the wild.

Despite the paucity of workers, the tools of biotechnology are being applied to *M. leprae*. At least three investigators are preparing MAbs as part of studies on the immunochemistry or antigenic structure of the bacterium (16).

TUBERCULOSIS

Aspects of Natural History

Tuberculosis is caused by the bacterium *Mycobacterium tuberculosis*, transmitted mainly by airborne droplets from person to person. There is no insect vector or animal reservoir. Tuberculosis remains a major threat to health in many parts of the world, causing several million deaths annually. Though basically a chronic respiratory disease, tuberculosis can spread into the cardiovascular, endocrine, and genital systems, and into the lymph nodes, bone, brain, kidneys, and other organs (406).

Incidence

A 1982 worldwide estimate put the number of tuberculosis cases at 11 million (265), with some 3.5 million new cases occurring each year (92). Tuberculosis is still a serious problem in most countries of the world. About 500,000 deaths are attributed to the disease each year.

Even in countries such as Canada and the United States, with highly developed coverage for diagnosis and treatment of tuberculosis available, a significant number of cases of tuberculosis are encountered each year. Often the disease is present among immigrants arriving from tuberculosis-prevalent tropical areas. From 1962 to 1975, prior to the current wave of immigrants to the United States from Indochina, the tuberculosis case rate among children under 14 in the United States decreased from 10.4 to 3.7 per 100,000, representing a 9-percent decline per year. But this downward trend has not continued, and the rate in 1984 was about the same as that of 1975 (281). In 1980, some 30,000 new cases of pulmonary tuberculosis were reported in the United States (70).

Interventions

In the past, control of tuberculosis was based on the identification, isolation, and treatment of patients with active disease, since these patients are the source of continued transmission and spread. More recently, with the advent of effective chemotherapy and reduction of active cases, developed countries with lower prevalences of tuberculosis have emphasized identification of newly infected persons via the tuberculin skin test and treatment of these individuals, in addition to identification and treatment of persons with active disease. Developing countries continue to emphasize identifying and treating patients with active disease, who account for most of the transmission. Tuberculosis is generally diagnosed by sputum microscopy or culture.

Treatment of tuberculosis patients consists of daily administration of one or more drugs in combination for 6 to 12 months. The drugs most commonly used include isoniazid (INH), streptomycin, para-aminosalicylic acid (PAS), pyrazinamide, and rifampicin. If the course of treatment is followed properly, cure rates can be as high as 100 percent (406). Because of the practical problems of long-term treatment, however, a 100-percent cure rate is seldom realized.

Unfortunately, some strains of *M. tuberculosis* have developed resistance to INH, the most effective drug available. Cases of INH-resistant tuberculosis are commonly reported in tropical areas from Asia to Latin America. Patients infected with resistant strains must take several chemotherapeutic drugs, and for a longer time period, before the infection is arrested.

A factor strongly contributing to the resistance problem is the high cost of rifampicin. This drug, when used with INH, is highly effective in controlling tuberculosis, but it costs about 400 times as much as INH (see ch. 9). Developing countries that cannot afford to use rifampicin in their treatment regimes often opt for other less expensive drugs. The short-term savings, are lost, however, because patients often do not complete long-term regimens. Partial treatment that does not eliminate the infection encourages the proliferation of drug-resistant organisms, as the most susceptible are killed off even with an incomplete course of therapy.

Despite the availability of a vaccine for tuberculosis, Bacillus Calmette-Guerin (BCG), there is considerable controversy as to its effectiveness. BCG vaccine is derived from a live, attenuated bovine tubercle bacillus, isolated from a single strain by the Pasteur Institute 50 years ago. BCG vaccination of uninfected persons can produce high resistance to tubercle bacilli, but the protection against tuberculosis has varied greatly in field trials. Since the vaccine was made years ago against only one isolated strain, it may not be capable of immunizing individuals against all currently active strains of *M. tuberculosis*. Nevertheless, some trials have shown very effective protection, and BCG vaccine is still recommended in high-risk areas.

Recent Progress

The tuberculin skin test is not an adequate predicter of infection or active tuberculosis, particularly for research purposes. The use of antigen probes and ELISA methods may be helpful in more accurately characterizing the state of an infection (16).

DIARRHEAL AND ENTERIC DISEASES

Aspects of Natural History

Diarrheal diseases, all of which are transmitted by fecal contamination of food and water, constitute a clinical syndrome of varied etiology. Such diseases are caused by a variety of viruses (primarily rotaviruses), bacteria (*Shigella*, *Salmonella*, *Cryptosporidium*, *Escherichia coli*, *Campylobacter*, and *Yersinia*), protozoa (*Entamoeba* and *Giardia*), and worms (*Ascaris*, *Ancylostoma*, and *Necator*), interacting in a complex fashion within the susceptible host. Diarrheal diseases are distributed worldwide.

Diarrhea is primarily a disease of infants and children. The great danger in diarrheal disease is the dehydration and subsequent shock caused by tremendous losses of fluids and electrolytes (salts). Severe dehydration is the most frequent cause of diarrheal deaths. Although treatment directed at the disease-causing organisms may or may not be effective, there now exists effective treatment for most cases of dehydration caused by diarrhea. Oral rehydration therapy (ORT) for diarrheal diseases is considered one of the most significant therapeutic advance in the past several decades (see *Case Study A: Oral Rehydration Therapy for Diarrheal Diseases*).

In 1980, WHO conservatively estimated that among children under the age of 5, 750 million to 1 billion diarrheal episodes occur yearly in Africa, Asia (excluding the People's Republic of China), and Latin America (324). Data from several developing countries indicate that children under 5 years of age in these countries typically experience four to eight diarrheal episodes annually (23,215,216). In contrast, infants in the United States and other developed countries experience one or two diarrheal episodes yearly. In some countries, up to 45 percent of all hospital visits during the months of highest diarrhea prevalence are due to childhood diarrhea, and case fatality rates as high as 40 percent have been recorded (249).

A comparison of death rates between children under 1 year of age in Latin America and North America is startling. For the United States and Canada, the mortality rate due to diarrheal disease among infants in 1979 was 21.9 deaths per

100,000 infants; for Latin America, it was 914.6 per 100,000. The Latin American figure is 40 times higher, which means almost 1 in 100 infants born there dies of diarrheal dehydration. Wherever the infant mortality rate exceeds 100 per 1,000 births, at least one-third of the deaths can be attributed to diarrhea (216,257).

Viruses

The complex of diarrheal diseases caused by viruses—rotaviruses, Norwalk-like agents, adenoviruses, astroviruses, enteroviruses, coronaviruses, calciviruses, and others, perhaps not yet identified—present extreme difficulties in diagnosis (15). Viral agents cause a significant amount of diarrheal illnesses in the tropics, but very little is known about any of them except rotaviruses.

Rotaviruses, the most frequently isolated group of viruses, have a worldwide distribution. They were first detected in humans in 1973. Rotaviruses, which are believed to cause one-third of all diarrheal disease in the world, may cause up to 40 percent of diarrheal disease in children in developed countries. Serologic studies have shown that by the age of 2, nearly all children have been infected with rotaviruses.

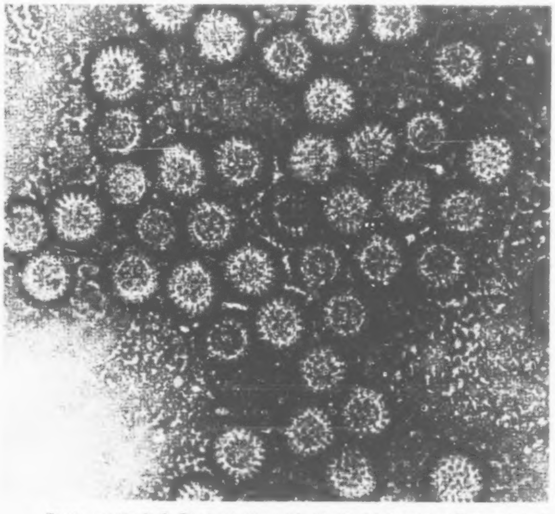


Photo credit: S. S. Raphael, "Lynch's Medical Laboratory Technology," W. B. Saunders Co., 1983. Reprinted by permission

Electron micrograph of a rotavirus, a major cause of diarrheal disease in children.

In Australia, Canada, the United Kingdom, and the United States, a large percentage (40 to 60 percent) of all children hospitalized with diarrhea are infected with rotaviruses; the percentage in countries like Guatemala and El Salvador is smaller (20 to 40 percent) (371,420). However, since the *incidence* of all diarrheal disease is much higher in developing countries than in developed countries, the toll due to rotaviral infection in these developing countries is actually much greater than the percentages indicate.

Growing recognition of the importance of rotaviruses emphasizes the need for further epidemiologic, clinical, and basic research. Rotavirus biology currently is studied at the National Institutes of Health, where each of the rotavirus genes has been cloned into bacteria and identified (117). Immunologically based diagnostic testing can be used for field studies of prevalence and incidence. Since a rotavirus vaccine exists for animals, a major objective now is to develop one for humans.

Bacteria

Numerous bacteria are known to cause diarrheal illness, and the list continues to grow as laboratory identification methods improve. In many areas of developing countries, the facilities needed to identify the agents responsible for diarrhea are not available. The question of causality is complicated by the wide variety of bacteria which live harmlessly in the intestine. This large "intestinal flora" confounds efforts to isolate and identify the important pathogens, among them enterotoxigenic (toxin-producing) *E. coli*, *Shigella* spp. (which cause dysentery), *Salmonella* spp. (which cause food poisoning and typhoid fever), *Vibrio cholerae* (which causes cholera), and *Campylobacter*.

At present, there is a great deal of concern about the apparent rise in antibiotic-resistant bacteria. Two separate factors may be responsible for the increase. One factor is the widespread misuse of antibiotics both in the United States and abroad, due to ineffective dosing of individuals, indiscriminate prescribing of drugs (regardless of the etiologic agent), and the availability of antibiotic drugs over-the-counter in many developing countries. As a result of this misuse, many intestinal bacteria, both pathogenic and nonpathogenic, are being exposed to antibiotics. Most intestinal bacteria are killed by antibiotics, but those bacteria that have some sort of resistance mechanism, often genetically determined, survive and are passed on.

The second factor is the ability of many intestinal bacteria to exchange genetic material among different strains, species, and even genera through the transfer of DNA. Bacteria that survive antibiotic exposure may transfer the genes that code for their resistance to previously susceptible bacteria, and these bacteria in turn incorporate them into their genetic material and pass them on to both pathogenic and nonpathogenic bacteria. Thus, in the past, a cholera infection might have been successfully treated with a few highly effective doses of tetracycline, but now larger doses of two or more other antibiotics may be required to combat the disease. Strains of *E. coli*, *Shigella*, and *Vibrio cholerae* have all been shown to be antibiotic resistant.

***Escherichia coli*.**—*E. coli* is second to rotaviruses as an important cause of diarrheal disease. For the American traveling abroad (or the foreign visitor touring the United States), *E. coli* is frequently the source of the infamous travelers' diarrhea.

Ironically, although *E. coli* is one of the most intensely studied of all organisms in the laboratory, little is known about its disease-causing abilities. Most strains are harmless inhabitants of the intestine, and only certain strains cause disease: enterotoxigenic *E. coli* produces toxins that result in excessive fluid production; enteroinvasive *E. coli* invades the cells of the intestinal wall; and enteropathogenic *E. coli* produces a toxin similar to that of *Shigella*, which causes diarrhea in infants. Investigators are currently studying the genetic basis for disease-causing properties of different *E. coli* strains.

***Shigella*.**—In 1981, there were 19,859 cases of diarrhea due to *Shigella* reported in the United States (366). Infections by *Shigella dysenteriae* result in bacillary dysentery, a serious and sometimes fatal disease. Most *Shigella* infections, however, are by species other than *S. dysenteriae* (e.g., *S. sonnei* and *S. flexneri*), and the symptoms are not as severe. *Shigella* is difficult to study because it colonizes only primates; thus, research on its pathology is very expensive. The genes coding for the attachment factors (which allow the organism to adhere to the intestinal wall) have been cloned and inserted into *E. coli*.

***Salmonella*.**—This genus is frequently divided into *Salmonella typhi*, the cause of typhoid fever, and the nontyphi species. In general, the nontyphi species of *Salmonella* cause self-limited gastrointestinal distress, although some species may cause bacteremia bacteria in the blood. The wide variety of symptoms associated with *Salmonella* infection, ranging from mild flu-like stomach upsets to severe food poisoning to typhoid fever, make the collection of accurate incidence and prevalence statistics almost impossible.

In 1981, there were 39,990 cases of salmonellosis food poisoning reported in the United States (367). Salmonellosis is usually associated with the consumption of contaminated livestock or poultry products. In that year, there were approximately half of the 510 cases of typhoid fever reported in the United States, about half of which were acquired during travel abroad. During the period 1970-80 while the incidence of typhoid fever in Latin America increased, the number of cases remained stable in North America and the Caribbean region (265).

Salmonella spp. usually cause endemic diarrhea, but they may cause epidemics. The need for improved sanitation and new antibiotics was brought forcefully home during a nontyphoid epidemic in Mexico in 1972-73, when it was discovered that the strain causing the epidemic was resistant to chloramphenicol, the drug of choice, and to other antibiotics (265).

Recently, researchers at the Walter Reed Army Medical Research and Development Command cloned *Shigella* genes into an attenuated live typhoid vaccine developed by a group of researchers in Switzerland. The result was a vaccine that produced immunity to both *Shigella* and *Salmonella* (126,423).

***Vibrio cholerae*.**—Cholera occurs in both endemic (in parts of China and India) and epidemic forms (in Asia and Africa). Because of improved sanitation in many countries, cholera epidemics are not as common as in the past, although one reached the U.S.S.R. as recently as 1970 (406). For unknown reasons, cholera epidemics have spared the Western Hemisphere.

The severe diarrhea characteristic of cholera is caused by the action of a bacterial toxin on the gut wall. One of the factors that makes cholera so virulent is the number and variety of transmissible genetic elements, which vary from one strain to the next. Researchers have been collecting cholera strains from endemic areas around the world, and recombinant DNA libraries, consisting of segments of genes from wild-type organisms and laboratory-grown strains, have been created. By studying and comparing the gene segments of toxigenic and nontoxigenic strains, scientists hope to pinpoint exactly which genes are responsible for cholera's virulence (16).

***Campylobacter*.**—Various species of *Campylobacter* are commonly the cause of sporadic diarrhea in developing and developed countries (45). Following rotavirus and enterotoxigenic *E. coli*, *Campylobacter* is the third most common cause of diarrhea in developing countries (297). *Campylobacter* also is a "frequent, cosmopolitan risk to travellers" (278). Epidemics sometimes affecting thousands of people, have been caused by *Campylobacter* contaminating unpasteurized milk, chicken carcasses, and water (194).

Protozoa and Other Agents

There are several protozoal diarrheal pathogens, among them *Entamoeba* spp., *Giardia lamblia*, and *Cryptosporidium* spp.

Giardiasis is now recognized as significant and ubiquitous throughout the United States, but is more prevalent in tropical countries. Outbreaks, such as occurred in Aspen, CO, and Rome, NY, have been well publicized (313). *G. lamblia* is a well-known hazard to camping and backpacking enthusiasts, who are becoming aware that wilderness water may not be as pristine as it looks. Many other less publicized outbreaks have occurred in preschool day care centers (400).

Cryptosporidium spp. are common diarrhea-causing agents in individuals whose immune systems are compromised, and they have also been found in some otherwise healthy individuals. *Cryptosporidium* was first described in 1907, but its oocysts (spherical egglike cells) were not recognized in animal feces until 1978. The importance of *Cryptosporidium* spp. has been appreciated only recently. Investigators using a simple diagnostic procedure developed in the last few years have estimated that the organism accounts for 1 to 4 percent of all cases of diarrhea in human beings (400).

Not much is known about chronic low-level enteric infections by bacteria such as *Yersinia* (the agent of plague). Multiple infections by several different pathogens frequently occur, making diagnosis difficult. One study of people living in a poor rural area of Panama with substandard sanitation showed that 90 percent of the 202 people examined were infected by one or more parasites, the majority of which were either *Ascaris lumbricoides* (roundworm), *E. histolytica*, or *G. lamblia* (88). WHO estimates there are at least 650 million people in the world with roundworm (ascariasis), 450 million people with hookworm (ancylostomiasis), 350 million people with amebiasis, and 350 million people with whipworm (trichuriasis) infections (318).

ACUTE RESPIRATORY INFECTIONS (ARIs)

Aspects of Natural History

ARIs are among the most important causes of preventable deaths in the world. They are a major cause of mortality among children under 5 and the elderly, sometimes exceeding the mortality rate due to diarrheal diseases. The ultimate consequences of an ARI depend on the organism(s) responsible for the infection and the patient's nutritional status and age.

All of the ARIs are aggravated by malnutrition and substandard living conditions, and the presence of other infectious diseases. A principal epidemiologic factor of ARI transmission is close, overcrowded conditions that promote inhalation of pathogens aerosolized by coughs, sneezes, and personal contact.

ARI agents include viral, bacterial, chlamydial, and mycoplasmal organisms, all transmitted to humans by airborne droplets. For medical purposes, ARIs are usually characterized as upper or lower respiratory tract infections (URTIs or LRTIs); then as community- or hospital-acquired; and then grouped by etiologic agent. Many of the same organisms cause infections in both the upper and lower respiratory tracts, however.

Incidence and Prevalence

Data about the frequency of ARIs are not generally available, making a discussion of incidence and prevalence of this important group of diseases difficult. Surveys in India, Guatemala, the United States, and the United Kingdom suggest that the incidence of ARIs is similar throughout the developing and developed countries, averaging four to eight separate episodes per year (175). However, mortality rates for ARIs in India are thought to be 30 to 75 times higher than those in the United Kingdom or the United States (175). What might cause inconvenient days out of work or school in a developed country might well kill a person in a developing country.

Some 12 percent of all deaths of children living in Africa, Central America, and Asia are attributed to ARI (289), and most of these children die of either pneumonia, bronchiolitis, or acute obstructive laryngitis (croup) (370). Among infants, mortality from ARI can range as high as 1,500 per 100,000 in areas of Egypt, Paraguay, and Mexico—a figure 30 times higher than in the United States and Canada (57)—or even higher to the 4,000 to 4,400 per 100,000 observed in areas of Bolivia and Brazil (370). The incidence of pneumonia, 70 to 100 cases per 1,000 per year, in children under 5 in developing countries, is double that of the United States. Among children who are suffering from malnutrition, almost half will also contract pneumonia during any given year (370).

WHO data, collected from 88 countries on five continents, representing a quarter of the world's population, reported over 660,000 deaths from ARIs in 1978 (35). Extrapolating this figure to the world population suggests that there are more than 2.2 million deaths from ARIs per year, a significant number of which could have been prevented. In addition to presenting a serious mortality risk for the very young and the very old, ARIs impose a tremendous social burden in terms of lost productivity and demands on the health care system by all age groups.

Viruses

Viruses cause most URTIs and some important infections of the lower respiratory tract. Most viral infections of the upper respiratory tract—infections with respiratory syncytial virus (RSV), adenoviruses, rhinoviruses, coronaviruses, and influenza, parainfluenza, measles, and Epstein-Barr viruses, for example—are self-limiting, eliminated by a healthy immune system. In weakened individuals, particularly children, who may be malnourished and have other infections, however, even normally benign URTIs can be life-threatening.

Measles is one of the few URTIs against which a highly effective vaccine exists. The vaccine's use is not universal, however, and measles remains a major cause of death among children in the developing world. Worldwide, 900,000 people, mainly children, died from measles in 1979 (392). The high death rate may be due to concurrent infections, or overcrowded living conditions, which result in heavy exposure of the susceptible individual to the virus whenever several members of the same household are infected (118).

In general, LRTIs cause more serious health problems than do URTIs, though they are caused by many of the same viruses. RSV, measles, and influenza are major causes of pneumonias, which often lead to death in developing countries.

Bacteria

Bacteria cause a host of infections in and around the upper respiratory tract including the sinuses, throat, tonsils, epiglottis, larynx, and trachea. Four types of bacteria are particularly significant causes of URTIs. *Corynebacterium diphtheriae*, is the cause of diphtheria, an infection of the pharynx. A second, *Bordetella pertussis*, is the cause of pertussis (whooping cough). Effective vaccines to prevent diphtheria and whooping cough are routinely given to newborns in developed countries and are included in WHO's Expanded Program on Immunization. Immunization is much more effective than treatment for these two diseases.

A third bacterium, *Streptococcus pyogenes*, commonly infects the pharynx, but is also the organism responsible for rheumatic fever. The fourth is *Hemophilus influenzae*, which causes ear infections and the more serious condition of meningitis in children.

Bacterial pneumonias (LRTIs) are a major cause of morbidity and mortality in developing countries, as they are in developed countries. *Streptococcus pneumoniae* is one of the most common causes of bacterial pneumonia affecting all ages. Other causes of bacterial pneumonia are *S. pyogenes*, *Staphylococcus aureus*, *H. influenza*, *Klebsiella pnemonia*, *E. coli*, and *Pseudomonas pseudomallei*.

Most bacterial ARIs respond to treatment with antibiotics. Important exceptions are many hospital-acquired pneumonias, which are often caused by drug-resistant organisms, a problem in developed as well as developing countries.

Other ARI Agents

Other less known ARI agents include *Chlamydia* spp. (congenital) and *Mycoplasma* spp., usually responsible for URTIs and atypical pneumonia among adolescents and adults. *Chlamydia* spp. appear similar to bacteria, but their size is close to that of viruses. *Mycoplasma* spp., a group of organisms which lack a rigid cell wall, are considered by some to be primitive bacteria.

Interventions

For most viral ARIs, there is no treatment other than symptomatic and supportive relief, though bacterial infections can be effectively treated with antibiotics. Vaccinations for measles, whooping cough, and diphtheria are effective and are promoted for vaccinating children under the Expanded Program on Immunization of WHO. However, use of the vaccines for these diseases is frequently limited by the cost of vaccines, the ability of the health infrastructure to maintain a vaccination program, and the problems inherent in maintaining a "cold chain" for certain vaccines. (Cold chain is the name given to the means for continuous refrigeration of vaccines from production to vaccinee.)

Measles is an example of an ARI which could, like smallpox, be eradicated by vaccination. Prior to the development of the measles vaccine in 1962, 481,530 cases were reported in the United States, 46 resulting in deaths. In 1979, after several years of widespread use of the vaccine, there were only 13,597 cases and only 1 death (366). In many developing countries, however, vaccinations against measles have been infrequent. Among children under 1 year of age in 1982, only 8.3 percent in Mexico, 15.9 percent in Bolivia, and 22.4 percent in Colombia (265) were immunized. Measles vaccine must be administered after maternal antibodies lose their effectiveness, but prior to the child's first exposure to a virus, in order for it to work—a critically short time span.

Vaccines against pneumococcal pneumonia (due to *S. pneumoniae*) and influenzas are available, but their usefulness in developing countries, is limited because pneumococcal vaccine is not very effective in children under 2 years old and influenza vaccines must be renewed periodically according to the currently prevalent strain. Because of the high cost of annually manufacturing a different strain of influenza vaccines, influenza vaccines are generally available only to high-risk groups.

Improved living conditions and access to health care are critical factors in controlling ARI, but field-based epidemiologic studies are also needed. ARI control is largely ignored in most developing countries. This situation is a function of several factors: difficulty in identifying the etiologic agents of ARIs, lack of effective treatment for many ARIs, and failure to define ARIs as tropical diseases, or to recognize ARIs as worthy of focused research.

The prevention and treatment of ARIs could be simplified through studies precisely identifying the important etiologic agents in different geographic areas and determining the risk factors that make ARI mortality so high. Practical management of ARI depends on differential diagnosis of viral from other bacterial, chlamydial, and mycoplasmal agents for which specific treatments are effective.

Biotechnology and ARIs

The tools of biotechnology are slowly being applied to the many viruses that cause ARIs in humans, but bacterial agents are being largely ignored.

ARI viruses are being isolated, identified, and characterized. A few laboratories are working on the molecular biology of influenza are studying mechanisms of immunity (76) as well as the effectiveness of immunization (271). All of the influenza genes have been identified and sequenced. Recently, some of the influenza genes have been cloned into the vaccinia virus used to inoculate against smallpox. Hamsters were inoculated with the vaccine and immunity was produced against both smallpox and influenza (319). There is hope that all the genes from the various types of influenza can eventually be cloned into the vaccinia virus to produce a single vaccine effective against every strain.

ARBOVIRAL AND RELATED VIRAL INFECTIONS

Aspects of Natural History

Arboviral infections constitute a large group of diseases caused by viruses (currently about 80 known in humans) defined by ecologic, epidemiologic, and clinical, rather than taxonomic, characteristics. The term "arbovirus" is a contraction of "arthropod-borne virus." Strictly speaking, arboviruses replicate in and are transmitted by arthropods (predominantly mosquitoes, but also ticks, sandflies, midges, and gnats) (a generalized arbovirus life cycle is shown in fig. 4-15). However, there are some arbovirus-like diseases whose vector is still unidentified (e.g., those caused by the Arenaviridae family) and some whose early epidemiologic profile incorrectly suggested arthropod transmission (e.g., Argentinian and Bolivian hemorrhagic fevers). These exceptions are noted, but for lack of a better characterization system, they are discussed here.

Arboviruses are widely distributed throughout all areas of the world and cause significant endemic and epidemic disease. Most arboviral diseases are infections of animals accidentally transmitted to humans (zoonoses), although epidemics of human-to-human transmission, via insect vectors, can occur.

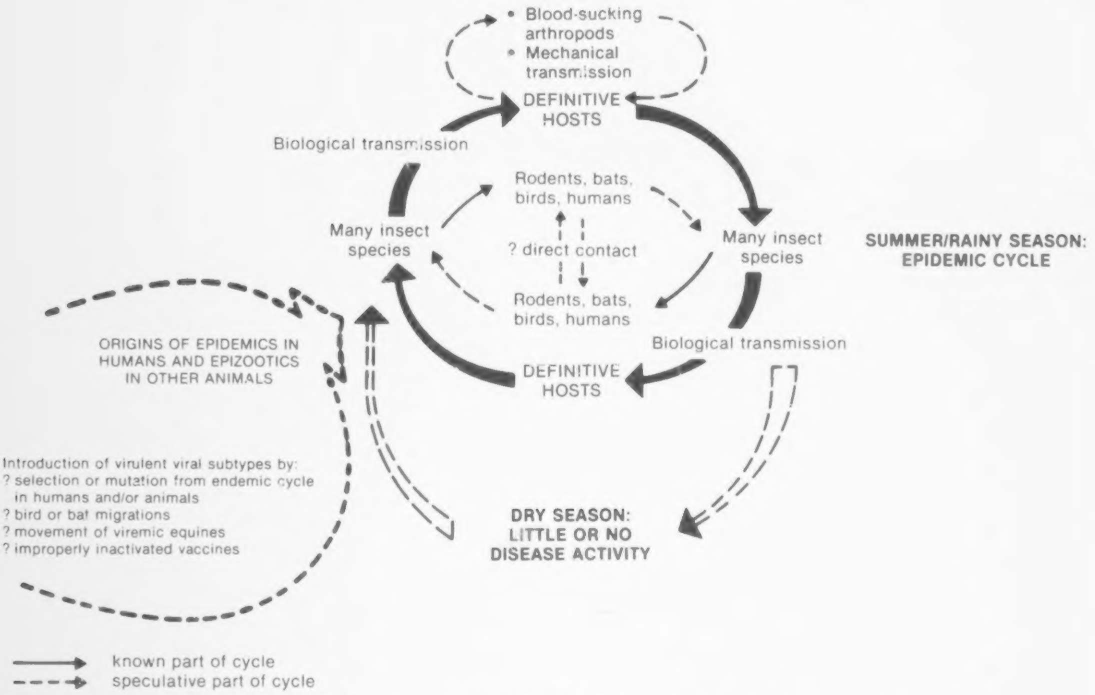
The number of known arboviruses has grown rapidly as the etiologic agents of many fevers and brain inflammations (encephalitis) have been identified and their transmission cycles elucidated. The major groups of arboviruses are classified by their biochemical and physical properties into the Togaviridae, Bunyaviridae, and Arenaviridae families.

The Togaviridae family is divided into two groups. There are about two dozen "alphaviruses" (formerly known as Group A), including the agents of Eastern, Western, and Venezuelan equine encephalitis frequently found in North and South America, Chikungunya of Africa and the Far East, and Sindbis and Semliki Forest viruses of Africa; and three dozen or so "flaviviruses" (formerly Group B), including the agents of yellow fever, dengue fever, Japanese B encephalitis and Saint Louis encephalitis (found in the United States). About a third of the flaviviruses are tick-borne and cause various febrile and hemorrhagic illnesses.

The Bunyaviridae family includes several hundred distinct viruses, among them the agents of California encephalitis, Oropouche fever of Brazil, Crimean hemorrhagic fever, and Rift Valley fever of east Africa.

The Arenaviridae are not arboviruses, although the possibility remains that arthropod vectors will someday be identified for the group. Included here

Figure 4-15.—Generalized Transmission Cycle of an Arbovirus


• Blood-sucking arthropods
• Mechanical transmission
DEFINITIVE HOSTS
Biological transmission
Rodents, bats, birds, humans
Many insect species
? direct contact
Many insect species
SUMMER/RAINY SEASON: EPIDEMIC CYCLE
Rodents, bats, birds, humans
Biological transmission
DEFINITIVE HOSTS
ORIGINS OF EPIDEMICS IN HUMANS AND EPIZOOTICS IN OTHER ANIMALS
Introduction of virulent viral subtypes by:
? selection or mutation from endemic cycle in humans and/or animals
? bird or bat migrations
? movement of viremic equines
? improperly inactivated vaccines
DRY SEASON: LITTLE OR NO DISEASE ACTIVITY
known part of cycle
speculative part of cycle

SOURCE: Adapted from G. T. Strickland, *Hunter's Tropical Medicine* (Philadelphia: W. B. Saunders Co., 1984), used by permission.

are some of the most deadly infectious agents known, such as the Lassa fever agent, as well as the more common lymphocytic choriomeningitis and various South American viruses, such as Junin (Argentine hemorrhagic fever) and Machupo (Bolivian hemorrhagic fever). Lassa and Machupo fevers are carried by rodents, but the rodent-to-human connection has not been made. Work with the more dangerous arenaviruses is restricted to the very few laboratories in the world with adequate containment facilities, among them the Fort Detrick facility of the U.S. Army in Frederick, MD.

Four basic types of clinical conditions are caused by arboviral infections. Two are generally benign and self-limited: 1) fevers of short duration, with or without a rash; and 2) painful joints and rashes of a short duration. Complications can develop from either of these two conditions, but they are the exception. The two much more serious clinical syndromes caused by arboviral infections are: 1) acute central nervous system disease usually with inflammation of the brain (encephalitis), ranging in severity from mild aseptic meningitis to coma, paralysis, and death; and 2) hemorrhagic fevers, with extensive hemorrhaging, associated with shock and high case fatality rates (liver damage and jaundice accompany these symptoms in yellow fever).

Yellow fever was the first arboviral disease of the tropics to be recognized, by Walter Reed, as a mosquito-borne disease. William Gorgas led the campaign to eliminate the disease from the Panama Canal Zone and from Cuba in the early 1900s. Further success occurred throughout Latin America. Although the virus is maintained in monkeys in the wild, yellow fever has not occurred in urban areas of Latin America since the 1920s.

Symptoms of yellow fever include rapid onset, high fever (103° F), headache, nausea, vomiting, and muscle pain. The disease in a population occurs in periodic cycles stretching over several years. The cycles depend on the buildup of nonimmune individuals in a population, who are then swept by an epidemic of the virus, leaving an immune population of survivors. Safe and effective vaccination of human populations near endemic jungle areas is one control strategy and provides immunity for at least 10 years. Surveillance of monkey populations and jungle mosquitoes by sampling for virus isolation is an important monitor, providing early warning of high levels of infection.

Yellow fever still remains a major threat in tropical America and Africa (see fig. 4-16), because the virus is maintained by transmission through a number of jungle mosquitoes, with monkeys and possibly certain marsupials serving as reservoirs. Recent research has demonstrated that passage of the virus from the female mosquito to the egg (transovarial transmission) occurs among the vectors of yellow fever. Thus, the mosquito may function not only as a vector, but also as a reservoir (100).

Cases of yellow fever in humans are associated with humans invading the jungle habitat. In recent years, however, an outbreak appeared in Colombia where there were no apparent known vectors or reservoirs, and in Trinidad where no cases had been detected for almost 20 years. The possibility of unknown reservoirs is of concern, as *Aedes aegypti*, the vector of yellow fever in the urban setting, remains abundant throughout the

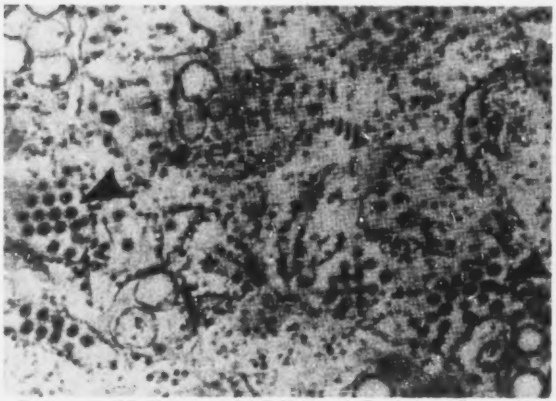
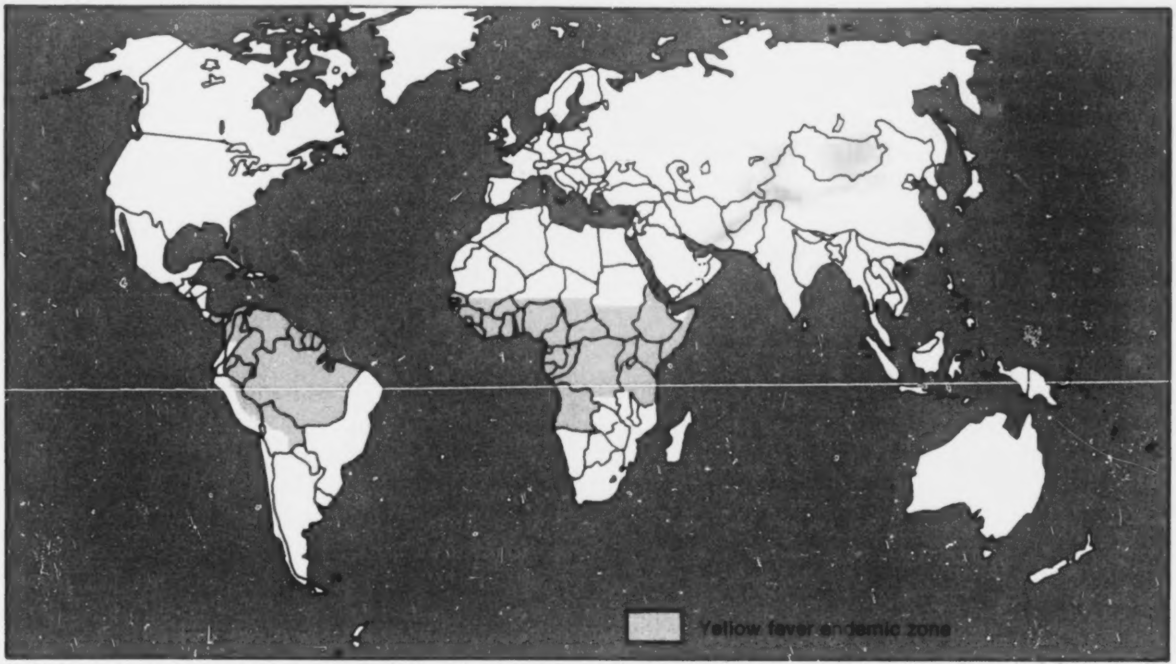


Photo credit: K.S. Warren and A.A.F. Mahmoud "Tropical and Geographical Medicine," McGraw-Hill Book Co., 1984. Reprinted by permission.

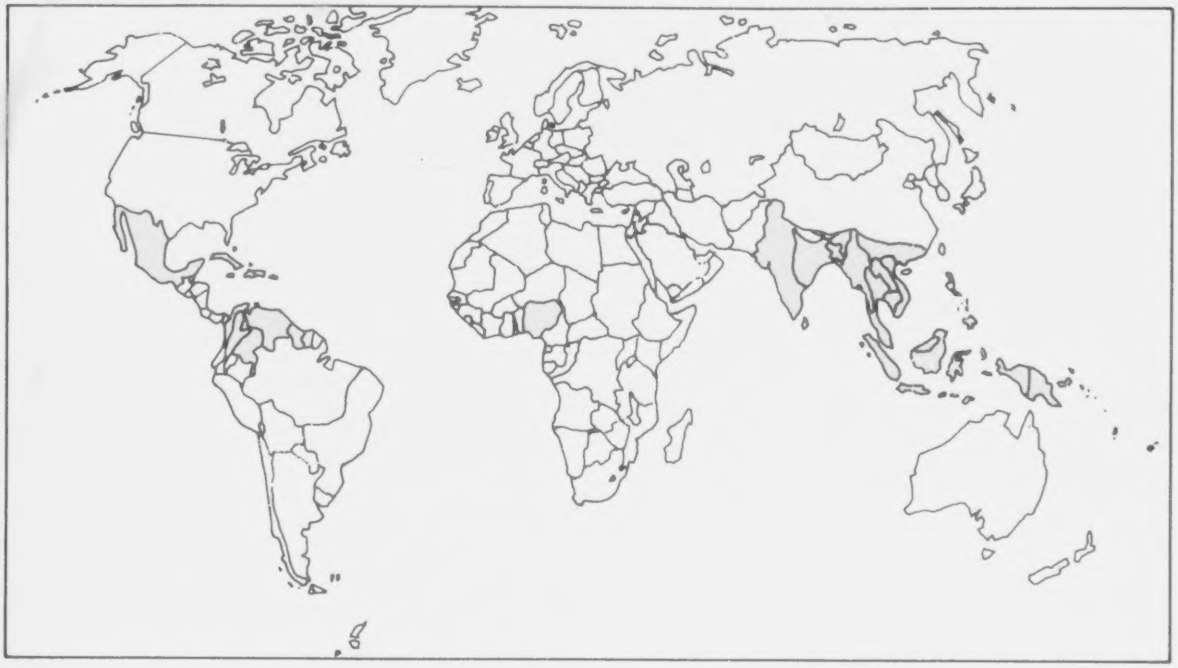
Electron micrograph of the dengue virus, agent of dengue fever.

Figure 4-16.—Geographic Distribution of Yellow Fever



SOURCE: U.S. Department of Health and Human Services, Centers for Disease Control, *Health Information for International Travel*, HHS Publication No. (CDC)840-8280 (Washington, DC: U.S. Government Printing Office, 1984).

Figure 4-17.—Geographic Distribution of Dengue Fever



▨ Dengue activity

☐ Potential for dengue activity because of presence of vector mosquito *Aedes aegypti*

SOURCE: World Health Organization, Geneva, Switzerland.

world (including the United States), posing a persistent threat of epidemic outbreaks in large population centers.

Dengue fever is a disease of worldwide distribution (see fig. 4-17) caused by four serotypes of the dengue flavivirus. The disease is usually mild, characterized by a rash resembling measles or scarlet fever, accompanied by generalized swollen lymph glands. Convalescence is long and distressful (406). Serious, sometimes fatal complications of dengue fever are dengue hemorrhagic fever and dengue shock syndrome. Dengue hemorrhagic fever usually affects children. In recent years, large epidemics of this virus have swept the Caribbean and Central America. In 1981, the first indigenous cases in the United States since the 1940s occurred. The virus is endemic to the Caribbean and is transmitted by mosquitoes of the genus *Aedes*, including the common urban mosquito *A. aegypti*, which is found as far north as St. Louis, MO.

Oropouche fever, found in Trinidad and Brazil, is emerging as an important arboviral disease because of its debilitating symptoms, which include anorexia, rash, and joint and muscle pain. The virus is transmitted by biting midges (*Culicoides* spp.) in urban and suburban areas. There is probably also a transmission cycle in forest animals away from populated areas, as in the case of yellow fever.

Rift Valley fever was first characterized in the Rift Valley of Kenya in 1931. Sandflies and mosquitoes are the suspected vectors, and while they have been shown capable in the laboratory, transmission has not been established in the field. Until 1977, Rift Valley fever was geographically limited to sub-Saharan Africa. Human fatalities were not known until the 1975 epizootic (epidemic among animals) in South Africa (104). Currently, the disease is present throughout Africa, but has not yet spread to other continents. In 1977-78, a widespread epizootic and epidemic of Rift Valley fever occurred in Egypt. Some 18,000 human cases were officially reported, and almost 600 people died of the disease (209). The disease in animals has a serious economic effect, causing cattle to abort and high fatality rates among newborn lambs. In humans, infection usually results only in mild dengue-like febrile illness, although it can result in severe ocular (inflammation of the retina and blindness), encephalitic, or hemorrhagic complications.

Lassa fever is endemic to some regions of West Africa. Symptoms include prostration, severe sore throat, tonsillitis, chest pain, and pneumonitis (inflammation of the walls of the air sacs in the lung). There is evidence that mild or subclinical infections occur, but among those hospitalized with Lassa fever, fatality rates range from 20 to 40 percent.

Research Needs

Laboratory techniques can identify arboviruses and define antigenically similar groups, but there is great geographic and climatic diversity in each serologic grouping. This situation emphasizes the complexity and challenge of arbovirus research and control.

There is no cure for arboviral diseases, only symptomatic and supportive relief. Early diagnosis of the agents responsible for potential outbreaks and epidemics identified down to the finest level possible, has three important values: 1) to differentiate ambiguous presenting symptoms; 2) to anticipate life-threatening complications, as in yellow fever and dengue fever; and 3) to target the vector, which then determines control strategies. Current control efforts rely on early identification of epidemic outbreaks of arboviral infections, allowing the institution of vector-control measures such as insecticide fogging. In many tropical countries, however, disease surveillance is not well developed.

5.

Strategies and Technologies for Controlling Tropical Diseases

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5.

Strategies and Technologies for Controlling Tropical Diseases

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INTRODUCTION

Throughout history, diseases have been brought under control by a range of direct and indirect measures. In most cases, disease control has been an unplanned bonus of economic and social development. The opportunities for controlling a disease either directly or indirectly depend on the relationships among the disease-producing organism, the environment, and humans. The more complex the relations, the more points for intervention, but not necessarily for success in controlling the disease. Experience shows that the most complex infectious diseases are, in general, the most poorly controlled.

In the developed world the control of infectious diseases has come about largely as a result of improvements in living conditions. Because of better sanitation, uncontaminated potable water, uncrowded housing, and an adequate diet, exposures to pathogens are less frequent and less intense, and people are better able to fight off severe infection.

Many recent declines in U.S. mortality rates are due to specific interventions, such as new vaccines and drugs, that are targeted at specific diseases (e.g., vaccines and antiviral drugs for hepatitis B). Three types of biomedical tools are used to control many of the infectious diseases of the developed world: 1) immunization techniques to prevent disease; 2) diagnostic technologies; and 3) therapy, including surgical and medical treatment. A fourth tool important to controlling many tropical diseases is vector control—i.e., control of the insects and other organisms that transmit disease-producing organisms to humans (e.g., mosquito vectors of malaria) or are otherwise necessary for the disease-producing organism to complete its life cyle (e.g., aquatic snails that serve as intermediate hosts for schistosomes).

These four tools are the primary direct measures that can be used to control diseases in individuals. Along with disease surveillance (which may involve screening segments of the population for signs of disease or immunity) and keeping track of vector populations and their infection rates, these tools are also the means for controlling infectious diseases in populations.

The remaining chapters of this report review the status of vector control (ch. 6), immunization (ch. 7), diagnosis (ch. 8), and treatment (ch. 9) for the tropical diseases of interest in this report: malaria, schistosomiasis, trypanosomiasis, leishmaniasis, filariasis, leprosy, tuberculosis, diarrheal diseases, acute respiratory infections (ARIs), and arboviral and related viral infections. Current research and promising developments are highlighted for each. This chapter provides background for the chapters that follow by laying out the strategies, using single and multiple interventions, for controlling tropical diseases.

Some gains in understanding tropical diseases have yet to be applied to any specific control measure. The methods of biotechnology (e.g., the use of recombinant DNA technology), for example, have led to new knowledge about the immunology of parasitic disease organisms. Although some of this knowledge may eventually be applied to the development of vector control, vaccines, diagnostic or therapeutic measures, it currently has no practical application. Biotechnology's contributions to advance in research toward disease control are brought to light in several chapters of this report. In the latter part of this chapter, some of the methods basic to biotechnology are described.

STRATEGIES FOR CONTROLLING TROPICAL DISEASES

Most developing countries of the tropics are still at the stage where increased investment in sanitation and clean water would have a tremendous benefit in the control of disease. Extending clean water and sanitation facilities to as many of the world's people as possible continues to be a goal of development agencies and international health groups. Although this report does not evaluate the success of such efforts, it is clear that large portions of the world's poor remain unserved. The tropical disease control measures in the biomedical spectrum, which are the focus of this report, will not solve the underlying problem of the lack of sanitation and clean water, which in many cases contributes to disease transmission. In many cases, however, these measures can protect against contracting disease and reduce morbidity and mortality due to disease.

Worldwide eradication is possible for very few diseases. Smallpox was eradicated through a worldwide vaccination program, and the eradication of measles may also be possible. For most of the diseases considered in this report, however, the most realistic goal at present is not eradication but control. Control means lowering morbidity and mortality to tolerable levels and containing the spread of disease.

Certain features of smallpox were essential to its eradication: 1) smallpox is transmitted directly from person to person, with no vector; 2) smallpox is transmitted only through fairly close contact with an infected person; 3) there are no animal reservoirs for smallpox; 4) there are no subclinical cases of smallpox (virtually everyone who is infected breaks out in the smallpox rash); and 5) smallpox develops within a relatively short time after exposure.

Most other major parasitic diseases—certainly the five included in the Special Program for Research and Training in Tropical Diseases (TDR)—depart from these characteristics. The parasites that cause malaria, trypanosomiasis, leishmaniasis, and filariasis are transmitted by vectors, and the parasites that cause schistosomiasis live part of their lives in intermediate snail hosts. The probability of eradicating vector-borne diseases by killing off all vector species or intermediate hosts appears slim. The story of the worldwide "malaria eradication campaign" waged by the World Health Organization (WHO) beginning in the 1950s has taught important lessons in that regard (see ch. 6).

Diseases that affect both humans and other animals pose special problems for control. The existence of animal reservoirs of disease means that even if all human cases are eliminated at any one time, the disease will still exist in animals and will eventually pass into humans once again. One strain of African trypanosomiasis is actually a disease of livestock, called nagana, which can be transmitted to humans. Yellow fever is an arboviral disease that exists in monkeys as well as humans. A highly effective, long-lived vaccine for yellow fever has existed for decades, and in many areas, transmission to humans has been largely curtailed. Because of the monkey reservoir, however, the absence of cases in humans does not allow discontinuing vaccination for yellow fever as was possible in the case of smallpox. Workers in development projects in Brazil, in which jungle is cleared for roads or agriculture, have been the victims of a type of yellow fever ("jungle yellow fever") in which the virus picked up by mosquitoes from monkeys is transmitted to humans. The control of yellow fever is quite good, but realistically, there will be no eradication.

The control of most of the other tropical diseases considered in this report is not as successful as that of yellow fever. The development of successful malaria vaccines, however, could put malaria control on a similar footing.

If eradication is not the goal, the options for disease control are varied, but it has been difficult to discover the most effective and most cost-effective mix of control measures. The "St. Lucia Experiment," for the control of schistosomiasis on the West Indian Island of St. Lucia, is one example of a successful research effort to compare the effectiveness and costs of several control measures (see box 5-A).

Box 5-A.—The St. Lucia Experiment

The St. Lucia experiment began in 1965 with a memorandum of understanding between the Government of St. Lucia and the Rockefeller Foundation to "cooperate in the study of matters affecting the health of the people of St. Lucia, and in seeking and applying measures to control disease, and, more specifically, schistosomiasis." In addition to the Rockefeller Foundation, the British Medical Research Council and Overseas Development Association sponsored the project, and the Edna McConnell Clark Foundation contributed funds.

Schistosomiasis is a debilitating disease caused by trematode worms that live in the human host's blood vessels. Eggs of the New World species (*Schistosoma mansoni*) are excreted in feces. The eggs hatch in water, enter certain types of snails, and eventually emerge from the snail in a form able to penetrate the unbroken skin of a person in the water. The link between feces-contaminated water and this disease is obvious.

Three interventions to control schistosomiasis were compared in different parts of the island of St. Lucia:

- controlling snails with molluscicides (chemicals that kill mollusks, the group of invertebrate animals to which snails belong);
- providing piped water to each house; and
- treating all infected people with drugs.

All three interventions were successful, and as a result, the incidence of schistosomiasis is now at an all-time low in St. Lucia.

The St. Lucia experiment began with a careful study of the social and economic factors affecting schistosomiasis. The St. Lucians' use of water and their beliefs about different sources of water affected, and in some cases, changed plans.

In the area of the island chosen for molluscicide treatment, snails were monitored for infection with schistosomiasis before spraying began. Rather than blanket spraying all waters, the approach was to spray only those areas with infected snails ("focal" spraying). In this area of the island, the schistosomiasis incidence rate (the rate of new cases) in children (up to 10 years old) fell from 22 percent in 1971 to 4 percent in 1974, and the prevalence (the rate of existing cases) gradually dropped over all age groups. In addition, the monitored snail populations showed a drop in infection from 3.9 percent in 1971 to 1.1 percent in 1974. By 1974, transmission was virtually nil. Those few people still excreting schistosome eggs were treated with chemotherapy; and focal spraying was continued at sites routinely used by people. In 1980, only 2 children of 700 were infected.

In the five villages of the island chosen for the second intervention, water was piped to each house, and a laundry and shower unit were built in each village. The rate of new infections fell, but transmission continued until an education campaign was instituted to decrease use of the river for washing. The rate of new infections in children fell from 30.6 to 12 percent between 1970 and 1975. Chemotherapy further reduced the rate to about 6 percent.

In the area of the island chosen for the third intervention, all people who were excreting schistosome eggs were treated with drugs. With fewer people excreting eggs, there was less contamination of water and a lower probability of infection. Three rounds of treatment brought new infection rates among children down to about 3 percent, both in areas where rates were high to begin with (23 percent) and in areas where rates were low to begin with (6 percent). Before the program, about 54 percent of the population was infected. After 3 years of chemotherapy, prevalence was down to 7 percent. The infection rate among snails also fell to almost zero.

Of the three interventions, chemotherapy produced the quickest, greatest decline in new infections, at the lowest cost. Annual per capita costs for the first 2 years were: $1.10 for chemotherapy; $3.70 for snail control; and $4.59 for water supply.

Each intervention had different advantages. Mollusciciding does not require the cooperation of all residents and is effective even if infected people move into the area. It is expensive, however, and getting more so as the cost of petroleum (the feedstock for molluscicide production) rises. Molluscicides kill aquatic life other than snails and cannot be used where people fish. Furthermore, in the absence of chemotherapy, this intervention does nothing for people already infected.

Piped water is fairly expensive and the equipment requires continued maintenance, but this intervention has the benefit of controlling more than just schistosomiasis. In the St. Lucia experiment, the villages with piped water had lower rates of infant diarrhea. Where water supply is part of an overall development effort, it should control schistosomiasis. Education about the benefits of using the clean water supply would probably increase the benefit.

Chemotherapy, the quickest and most effective single intervention, requires continued participation and cooperation from the community. Surveillance, particularly for immigrants from infected areas, must be kept up as well. And even though chemotherapy is relatively cheap, it still will be too expensive for many countries.

The St. Lucia experiment illustrates two important points. First is the encouraging fact that disease can be controlled successfully through several different methods. Second is the focus on *control*, rather than eradication. Maintenance measures to keep transmission to a minimum at a relatively small cost have been recommended to the Government of St. Lucia by the St. Lucia study team.

SOURCE: J.A. Cook, P. Jordan, and K.S. Warren, "The St. Lucia Experiment and the Evolution of a Contemporary Global Strategy for the Control of Schistosomal Disease," typescript, Rockefeller Foundation, New York, 1981.

TOOLS FOR CONTROLLING TROPICAL DISEASES

General Measures

The infectious diseases that killed most Americans at the turn of the century are now largely under control in the United States. Improved sanitation, the presence of adequate nutrition, and higher levels of education claim much of the credit. Those three conditions are not met in most developing countries.

Providing a secure, clean water supply for drinking and washing, and successfully educating people in how to use it, can reduce the transmission of diseases such as schistosomiasis (which is transmitted to humans through contact with water in which infected snails live) and diarrheal diseases caused by water-borne and food-borne pathogens. Sanitary disposal of human and animal waste further reduces the chance of spreading disease. In southern Africa, south Asia, east Asia, and the Pacific, less than half the population has access to a secured water supply or excreta disposal. In general, access is much greater in urban than in rural areas. In India, for instance, 80 percent of the urban population, but only 18 percent of the rural population, has water supplied. In Ethiopia, the corresponding figures are 58 and 1 percent (412).

Adequate nutrition buoys functioning of the immune system, increasing the individual's resistance to contracting diseases and his or her ability to fight a disease once contracted. Nutrition is particularly critical for pregnant women and infants.

Education, particularly of women, has beneficial effects on maternal and child health. The World Bank estimates that for every year of maternal education, infant mortality rates drop by nearly 1 percent (412). Although the exact sequence of events has varied around the world, in general, birth rates drop as the level of health increases. Fewer births lead to healthier children and women, and, in the long run, can help to improve nutrition.

Chagas' disease (American trypanosomiasis) could be controlled through the application of present-day knowledge. In Panama, for example, the Corozal palm (*Schelia* spp.), which provides thatch for rural dwellings, has been found to be a mini-ecosystem, harboring both reduviid bug vectors and mammalian reservoirs of *Trypanosoma cruzi*. If houses were constructed with corrugated metal or adobe tile roofs rather than thatch, then human contact with the reduviid bug vector of the disease could be limited considerably. Any attempts to control the reduviid bugs by insecticide only must be continuous, because the mammalian reservoirs of Chagas' disease provide a steady supply of *T. cruzi* parasites ready to infect the next generation of reduviid bugs that feed on them.

Vector Control Technologies (see ch. 6)

Many important tropical diseases, including malaria, trypanosomiasis, leishmaniasis, filariasis, and arboviral infections, are transmitted to humans by mosquitoes or other arthropods (e.g., ticks, sandflies, midges, and gnats). One, schistosomiasis, requires a second host, a freshwater snail, for the parasite to complete its life cycle. Control of vectors and snails in endemic areas is one way to control the transmission of these diseases.

Before about the middle of this century, most vector control efforts used mechanical approaches such as catching insects, removing breeding sites, or setting up physical barriers to the vectors' migration (e.g., by clearing strips of forest). Most vector control activities today use pesticides. The pesticide era began with the introduction of DDT (dichloro-diphenyl-trichloro-ethane) after World War II and has had some significant accomplishments. It also has taught that insects have a great capacity to evolve pesticide-resistant forms. In many parts of the tropics, stronger and stronger pesticides have been used against ever more resistant insects.

Other vector control strategies include biological control measures (e.g., the introduction of predators or pathogens of vectors) and environmental control measures (i.e., altering the environment to eliminate conditions necessary for the vectors' survival). The use of a combination of measures to control vectors is generally referred to as "integrated pest management" (IPM).

Immunization Technologies (see ch. 7)

The purpose of immunization is to prevent an individual from getting a disease if exposed to the agent that causes the disease. Vaccines are the most important tools of immunization. The functional components of vaccines are "antigens," substances that cause the body to marshal its own

Photo credit: Dr. Robert Edelman, National Institutes of Health

Irrigation ditches are frequently infested with the snail intermediate hosts of schistosomes.

immune system by activating one or more types of immune system cells. Some cells may be programmed to physically attack disease organisms and others are primed to produce "antibodies," substances that act to neutralize or kill the disease organism should the organism invade the body. Less important than the active immunity induced by vaccines is a passive immunity produced in an individual by the transfer of antibodies in blood serum from other, actively immune individuals.

All the human vaccines in use today are vaccines for bacterial and viral diseases. There are currently no vaccines for human parasitic diseases, but there is a great deal of research activity toward their development. The furthest along is a vaccine against one stage of the malaria parasite *Plasmodium* (see *Case Study B: The Development of a Malaria Vaccine).*

Vaccines benefit both the individuals immunized by them, and the community as a whole. Even if every person in the community is not vaccinated, if a high proportion are vaccinated or have acquired immunity from having had the disease, the nonimmune members will also be protected because there are not enough susceptible people to sustain disease transmission. This phenomenon is called "herd immunity."

In the United States most, but not all, children are immunized against measles, mumps, rubella, poliomyelitis, tetanus, diphtheria, and pertussis. In general, immunization levels are high enough to produce herd immunity. Outbreaks do occur, however, particularly outbreaks of measles. Investigations usually reveal that a critical mass of unvaccinated children existed in the community, and at least one was exposed to a case of measles. Although there are seldom deaths associated with measles in the United States, that is not the case in the developing world.

Immunization programs are potentially the most effective means of disease control for most infectious diseases, but they require a long-term commitment of public health resources to be successful. The initiation of vaccine programs requires careful analysis of the health, social, political, and economic conditions of the population to be immunized. (Table 5-1 lists the biologic considerations for using a vaccine against a tropical disease.) Since 1974, WHO has been the major promoter of childhood immunization, through its

Table 5-1.—Considerations for Use of a Vaccine for a Tropical Disease

Regarding the target organism:
- Complexity of antigenic structure
- Multiple stages of the organism's life cycle
- Accessibility of infection site within the host
- Occurrence of different species, geographic strains, local and temporal variants
- Inherent genetic variability of organisms
- Possibility of genetic selection for vaccine resistance

Regarding the vaccine:
- Immunogenicity of antigen: efficacy and effectiveness
- Use and type of adjuvant or diluent
- Possible undesirable side effects
- Targeted at one or several variants of the disease organism
- Separate administration or combined with other target vaccines
- Storage requirements (e.g., refrigeration and shelf life)

Regarding the vaccinee:
- Age
- Health status, including pregnancy
- Degree of possible resistance
  —due to genetic or ethnic factors
  —due to prior exposure or acquired immunity
  —due to current infection
- Protocol; type, number, and time of other immunizations
- Access to prophylactic and therapeutic drugs
- Route, mode, dosage, and number of inoculations
- Measurement of immune response
  —comparison with age-matched groups in developed countries
  —monitoring for side effects or adverse reactions
- Degree and duration of protection
  —from infection
  —from pathologic effects of disease

Regarding the vaccine trial:
- Randomized selection of vaccinee and control groups
- Characteristics of placebo used in control group
- Availability of baseline disease incidence and prevalence data
- Ethical aspects
- Training of field teams and standardization of procedures
- Site selection: number and distribution of trial populations
- Identifying populations at risk but not already infected or immune
- Determining exposure to and risk of infection by target organism
- Statistical validation of data; required number of participants
- Presence of related or competing organisms
- Length of followup
  —for safety
  —for efficacy
- Coordination with other public health programs—effect of vector control or other simultaneous projects
- Source and adequacy of funding

SOURCE: P. Basch, "The Role of Biotechnology in Tropical Disease Research," contract report prepared for the Office of Technology Assessment, U.S. Congress, Washington, DC, 1984.

Expanded Program on Immunization (see box 5-B).

Diagnostic Technologies (see ch. 8)

Diagnostic technologies serve several purposes:

- to determine pertinent characteristics of an individual's disease, so that appropriate treatment can be given;
- for public health reasons, to determine the prevalence of pathogenic agents in populations; if control measures (e.g., a vaccine) are begun, determining the prevalence of such agents is important to allow evaluation of the success of the measures; and
- for research purposes, to find out about the ranges of diseases affecting a population and the immune status of populations, for instance.

A wide range of biochemical tests supplements the observational abilities of medical personnel in diagnosis. Many of these diagnostic tests rely on reactions between antigens and antibodies and have radioactive or color markers that signify the test result. Recombinant DNA technologies are contributing to diagnostics in several ways, including the production of pure reagents for tests and the production of "DNA probes," which recognize the genetic material of disease-producing organisms.

Therapeutic Technologies (see ch. 9)

The object of therapy is to improve the lot of a person with disease. In the best case, therapy involves ridding the person of disease, but for many conditions, symptomatic relief is equally important. This report concentrates on drug treat-

Box 5-B.—WHO's Expanded Program on Immunization (EPI)

WHO's Expanded Program on Immunization (EPI) began in 1974 with a goal of immunizing all children against diphtheria, pertussis, tuberculosis, measles, tetanus, and poliomyelitis (272).

EPI is an operational attempt to apply safe and efficacious vaccines that already exist. Diphtheria, pertussis, polio, and tetanus vaccines have been available for many years and are recommended for widespread use. The efficacy of tuberculosis vaccine (Bacillus Calmette-Guerin vaccine) is more controversial (see ch. 7). Measles vaccination has also been controversial because of the variable seroconversion rate (percentage of vaccinated individuals who later have measurable antibodies) and protection rates (percentage of vaccinated individuals who do not get disease) obtained in developing countries. Measles vaccine is heat labile and not as effective as desirable in infants under 1 year of age.

Even with efficacious and safe vaccines, however, EPI has encountered problems of cost, logistics, and acceptance by the public, as well as strictly biomedical problems of immunizing malnourished populations already infected with other pathogens. A critical problem, and one that impedes EPI in many countries, is the need for a reliable "cold chain," the means to refrigerate vaccines from manufacture to use. In remote rural areas, the opportunities for the cold chain to break down increase, and with the breakdown, a damaged vaccine becomes more likely.

In countries that, for whatever reasons, cannot maintain an immunization campaign over the long run, vaccination programs may actually exacerbate disease rather than avert it. For diphtheria and polio, in particular, followup and regular boosters are necessary. Individuals who are vaccinated as children but not followed up as adults may still be susceptible to these diseases, potentially more severe than if they had occurred in childhood.

A technical problem is that the effectiveness of vaccines used in EPI may be impaired by the use of other health care measures. Chloroquine given for malaria prophylaxis, taken for long periods of time, can reduce the response to vaccines.

Even if new vaccines are developed, their impact on major public health problems in the tropics will still be problematic. Major questions that remain are whether the vaccines will work in large heterogeneous populations who are malnourished and often carrying other infections, whether the vaccine will be cheap enough and feasible to use (stable enough, easily administered), and what side effects will be encountered.

ment; it does not consider surgical treatment, because surgery plays a relatively minor role in the treatment of most tropical diseases.

Drugs to treat most bacterial infections exist, though bacteria in many cases are resistant to one or many drugs. Drugs to treat helminthic (worm) infections also exist, but these are generally less effective and less safe and require longer periods of treatment than do drugs against bacterial infections. The development of drugs to treat viral infections is just beginning. The antiviral drugs currently available are not commonly used against tropical diseases.

Genetic Tools

The science and industry of present-day biotechnology—the use of recombinant DNA techniques and other sophisticated genetic tools—are outgrowths of the ideas and experiments of a 19th century Austrian monk. In 1865, Gregor Mendel first described the concept that traits of higher organisms are transmitted from one generation to the next as discrete units. Although Mendel's work was unrecognized and unappreciated for half a century, the science of genetics had been born. For decades, the units of heredity, genes, were considered to be vaguely defined units or particles riding in some sort of linear order upon the chromosomes of animals and plants. Beads on a string were used as a popular analogy.

In 1953, Watson and Crick suggested a structure for the precise chemical makeup of Mendel's hypothetical hereditary units. The now familiar double-helix structure of deoxyribonucleic acid, or DNA, is at the core of modern molecular genetics, for it provides the framework of our understanding of the functioning of the genetic material and the basis for manipulating it.

The DNA molecule consists in part of an invariant "backbone" made up of two coiled strands of repeating sugar (deoxyribose)-phosphate components. Each sugar has attached to it one of four chemical bases. These four bases—adenine (A), cytosine (C), guanine (G), and thymine (T)—in certain combinations form bridges, something like the rungs of a ladder, across the paired helically coiled deoxyribose-phosphate strands. An A must pair with a T, in either order (A-T or T-A); and a C must pair with a G. Therefore, given a sequence of bases on one strand of a DNA molecule, say A-T-A-C-G-T, one can deduce the complementary sequence, in this case T-A-T-G-C-A, which must be attached to the other strand.

In the synthesis of new DNA from existing DNA, a process known as "replication," the two strands of the DNA molecule separate, each serving as a template. In the process of "transcription," the DNA strand serves as a template for a strand of a slightly different nucleic acid known as RNA, which in turn codes for the synthesis of proteins (see 357 and 361).

Recombinant DNA Technology

In 1973, Cohen, Chang, Boyer, and Helling reported the first in vitro production of DNA molecules that were "replicated" when transferred into living organisms. The details of recombinant DNA work are extremely complex and constitute the subject of many thousands of technical publications (e.g., 187, 132, 224, and 397).

Recombinant DNA technology is the term used for methods to transfer segments of genetic material from one organism to another, to replicate it, and to use it to make chemicals (see box 5-C). Recombinant DNA technology represents an extremely powerful and precise set of tools with unknown, but certainly great, implications not only for tropical medicine but for many aspects of life in the future. A widely acclaimed use of recombinant DNA technology is for the production, in commercial quantities, of materials such as human insulin, interferons, or growth hormone. An application of cloned complementary DNA or RNA (see box 5-C) is in locating a viral, bacterial, or parasitic DNA sequence within a host organism. These nucleic acid hybridization probes promise to be useful in molecular diagnosis of disease.

Monoclonal Antibodies (MAbs)

MAbs are homogeneous antibodies derived from clones of a single cell. To understand MAbs and the hybridomas that produce them, it is necessary to go back to the work of early immunologists.

Box 5-C.—Recombinant DNA Technology

Recombinant DNA technology is a process whereby pieces of DNA from different sources, as different as humans and bacteria, are joined together. The basic method for recombining DNA follows.

1. Start with a, b, or c:
   a. DNA is extracted from an organism. Within the total DNA (the genome) is the genetic information for making all of the components and characteristics of that organism. The trick is to pull out from all that DNA the gene of interest, which probably constitutes only 1/1,000th or 1/10,000th of the total.
   b. A reversal of the transcription process (in which DNA is a template for RNA) is used to make a particular piece of DNA. RNA is usually extracted from cells that are actively producing the desired material. Through the use of a special enzyme called reverse transcriptase, the strand of RNA is used as a template on which a segment of single-stranded DNA is synthesized. Because the structure of this new DNA strand is complementary (like the relationship between a photographic negative and a print made from it) to the structure of the RNA template, the newly formed DNA is called "complementary" DNA. The DNA and RNA strands of the "hybrid" molecule are separated and the DNA strand used as a template with the enzyme "DNA polymerase I" to synthesize a double-stranded DNA configuration.
   c. If the sequence of bases in the desired DNA is known, chemical methods are used for the direct synthesis of DNA. These methods are limited to "shorter" pieces.
2. The extracted DNA (from 1a) or complementary DNA (from 1b) is cut into pieces by use of restriction enzymes (restriction endonucleases) that cleave the DNA or complementary DNA molecule at certain specific sites. Restriction enzymes have been extracted from more than 200 different strains of bacteria and cleave the DNA molecule at any of more than 90 known sites, unique for any particular enzyme. The cleavage site is determined by the sequence of constituent base pairs. One of the most commonly used restriction enzymes, called "Eco RI" and derived from *Escherichia coli*, cleaves the double-stranded DNA molecule in such a way as to leave overlapping cohesive or "sticky" ends. The resulting segments may contain part of a gene, a whole gene, or several genes depending on the configuration of the particular DNA molecule being cleaved. If complementary DNA was the starting material, it is already enriched for the genes encoding for production of the particular material made by the cells from which the RNA was extracted.
3. The pieces of DNA are inserted into a "vector." [This borrowed term is perhaps an unfortunate selection—the DNA vector has nothing to do with an insect vector of disease. The idea is that the vector transmits something to a new site.] Insertion is accomplished by cutting the vector's own DNA with restriction enzymes and then splicing the fragments of foreign or "passenger" DNA between the cut ends. Vectors are introduced into bacterial cells in which they replicate hundreds or thousands of times. Each copy of the vector contains one copy of the "passenger" DNA. In this way, "clones" of DNA are produced.
   A vector may be:
   a. A "phage" (=bacteriophage), which is a virus that parasitizes certain bacteria. Phages normally grow by injecting their DNA into the bacterium. The phage DNA takes over the replication machinery of the bacterium and makes many copies of itself and ultimately new phage particles which can then infect additional bacteria; or
   b. A plasmid, which is a circular DNA molecule that normally occurs in many bacteria. Many carefully engineered plasmids that contain information necessary for replication, as well as sites for the insertion of segments of foreign DNA and genes useful in later selection processes are available. Because plasmids are smaller than the bacterial chromosome and replicated independently of it, a single cell can contain many plasmids.
4. The passage of the vector through bacterial hosts produces millions of new vectors. A number of clever methods can be used to sort through the new vectors to select the vector that contains the desired sequence of DNA. There may be very few, if any, of them: most

host cells will have no recombinant DNA, or will have fragments without immediate interest to the investigator.

5. Once a clone of the desired DNA is obtained, that DNA is introduced into cells (usually bacteria or yeast) where it directs the synthesis of the wanted product. One drawback of the most commonly used bacterial host, *E. coli*, is that this host may make, but may not release, the product whose manufacture is directed by the foreign DNA segment. Additional problems with this host are the ability (at least potentially) of some strains to produce a substance toxic to the human intestine, and the possibility of mutation and loss of the newfound capability. To circumvent these problems, many laboratories are using yeast cells as hosts, because yeasts in general are genetically more stable than bacteria, and there has been much more experience with yeasts in large-scale fermentation. Yeasts are also able to secrete proteins and to synthesize some complex materials beyond the ability of *E. coli*. Various other hosts have been or may be used.
6. Host cells exhibiting the desired characteristics are isolated in pure culture. They can be subcultured repeatedly and their products harvested.

SOURCE: P. Basch, "The Role of Biotechnology in Tropical Disease Research," typescript, contract report prepared for the Office of Technology Assessment, U.S. Congress, Washington, DC, 1984.

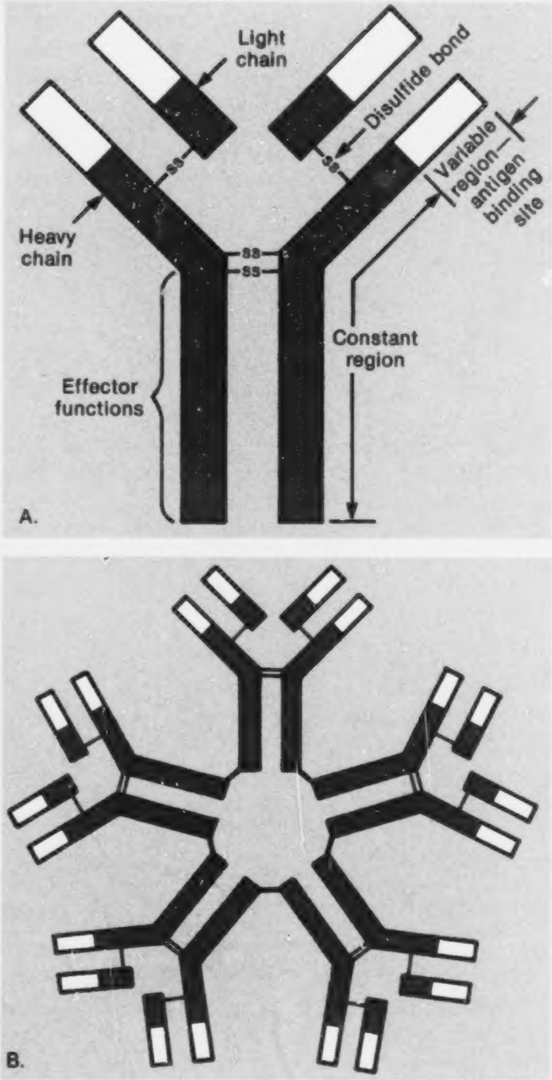
Investigators had observed around the turn of the century that in response to the natural or intentional introduction of foreign materials ("antigens," animals produce specific substances which circulate in the blood. These responsive substances became known as "antibodies," and early researchers such as Paul Ehrlich believed that the antibody and its inducing antigen fit together physically like a lock and a key.

Various theories of antibody formation and action were proposed by many workers, but it was not until 1959 that the Australian immunologist Sir Macfarlane Burnet conceived the theory of clonal selection. Burnet's idea, which led to a Nobel Prize, was that a great variety of quiescent but potentially antibody-producing cells exist in the normal human or animal, each with the inherent capability of secreting one specific kind of antibody. These quiescent but potentially antibody-producing cells are now known to be a certain type of white blood cells, which are called B-lymphocytes. According to Burnet's clonal selection theory, the introduction of any particular antigen into the body causes only those cells preadapted to it to become stimulated and to proliferate and differentiate into plasma cells that release specific antibody in measurable amounts. The many cells created by the repeated division of the originally stimulated cell are "clones," whose secreted antibody mixes in the bloodstream with the products of all other clones of antibody-producing cells.

The structure of antibodies is now known with great precision. Although there are several subtypes of antibodies, their basic composition and behavior is quite similar. As shown in figure 5-1, an antibody molecule has two symmetrical halves, each containing one "heavy" and one "light" protein chain. Each of the four chains contains a constant region (for certain generalized functions), and a variable region (on which are located the antigen-binding sites that determine the antibody's specificity).

For decades, immunologists had obtained antibodies for their research by the inoculation of animals, usually rabbits, with antigen. The antigenic dose could be as simple as a single purified protein, or complex as a whole-organ extract. After some time, the rabbit was bled, its blood allowed to clot, and the clear serum, containing the antibodies, was collected. At that point, the problem was always how to separate the particular antibody of interest from the mixture of very similar materials in the rabbit's blood serum. Scientists devised various chemical precipitations and immunologic binding methods to try to fish the desired antibody out of the complex "polyclonal" pool. Many problems were encountered in these procedures.

Figure 5-1.—Structure of Antibody Molecules



A. The basic unit of all antibodies is a protein molecule made up of two "light" and two "heavy" chains joined by disulfide bonds. Foreign antigens are recognized by regions at one end of the molecule (variable regions). Other parts of the molecule are specialized for "effector functions" that aid in deactivating and removing the foreign antigen. Antibodies in the immunoglobulin classes IgG, IgD, and IgE exist as single monomers.

B. Antibodies belonging to immunoglobulin classes IgM and IgA exist as molecules with multiples of the basic unit. IgM (in diagram) is a pentamer (5 units). Different types of IgA exist in monomers, dimers (2 units), and trimers (3 units). The units are joined by disulfide bonds.

SOURCE: Office of Technology Assessment, 1985.

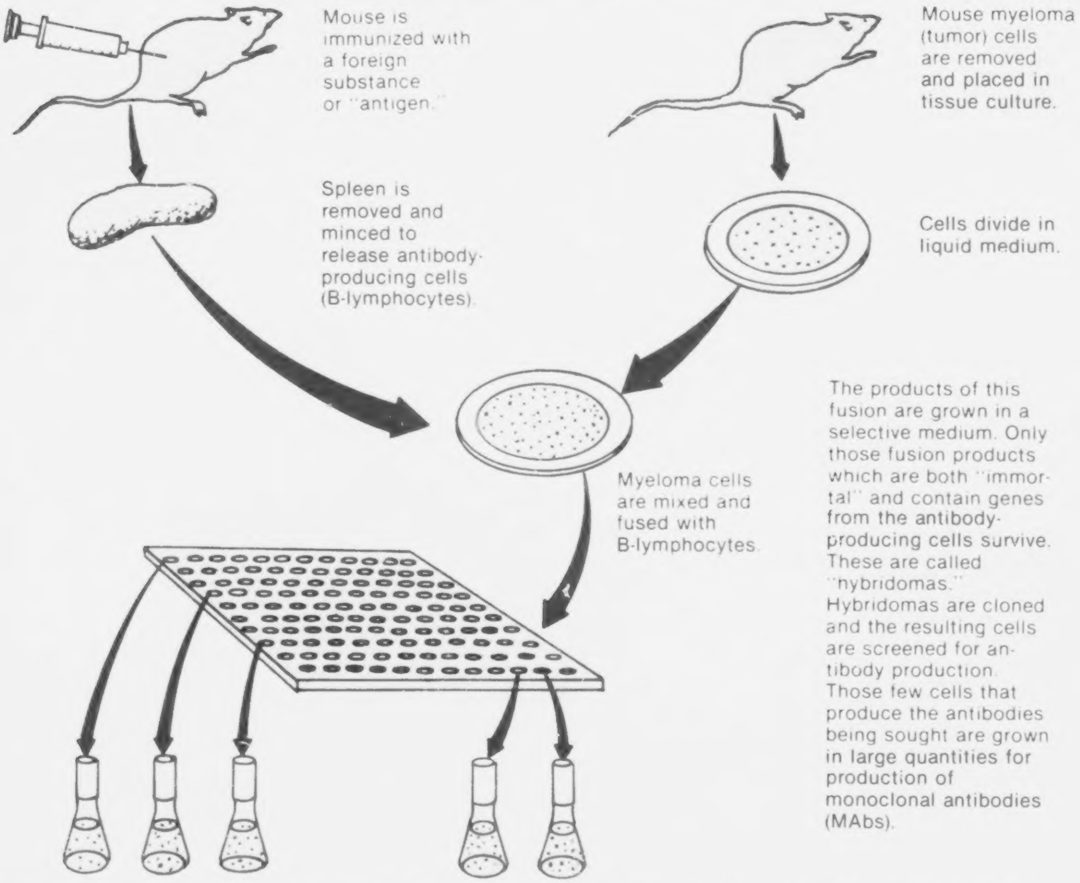
One problem was that complex antigens, as large molecules, usually bear several different structural antigenic determinants called "epitopes," each of which is recognized by distinct antibody-producing B-lymphocytes. The inoculation of a complex antigen results in the production by the immune system of several different antibodies, each of which is specific to a portion of the same antigen. Conversely, several distinct antibodies may combine with the same portion of the antigen. An additional complication is that rabbits, even from closely inbred lines, vary in their individual ability to recognize and respond to particular antigens: the antibodies produced are sometimes heterogeneous and vary from rabbit to rabbit or even within the same animal over time.

Scientists' inability to obtain a uniform, reproducible, and above all, specific product hindered biomedical research until the invention of hybridoma technology in the mid-1970s. This invention, by Georges Kohler and Cesar Milstein at Cambridge University in England, was a landmark in the history of immunology.

For some time, immunologists had attempted to isolate and clone antibody-forming B-lymphocytes in artificial culture media, but these cells normally have a limited life span and clones cannot be established. Malignant tumors of the immune system called "myelomas" are capable of continuous proliferation in cultures, but cultured myeloma cells produce little or no antibody. Kohler and Milstein devised a method for forcing the recalcitrant myeloma cells to produce specific antibodies in quantity. Their work, published in 1975, has led to an explosion of subsequent studies. What Kohler and Milstein did was to combine an immortal but nonsecreting myeloma cell line with a secreting but nonculturable B-lymphocyte in order to produce "chimeras" having the desirable properties of both parental types. This is the "hybridoma" (=hybrid myeloma) so commonly used today. Hybridomas and MAbs have been reviewed several times (e.g., 6), and step-by-step instruction manuals for their preparation are available (206,437).

The method for preparing MAbs is shown in figure 5-2. First, a mouse is immunized with an antigen and permitted some time to respond.

Figure 5-2.—Preparation of Monoclonal Antibodies (MAbs)



Mouse is immunized with a foreign substance or "antigen."

Spleen is removed and minced to release antibody-producing cells (B-lymphocytes).

Mouse myeloma (tumor) cells are removed and placed in tissue culture.

Cells divide in liquid medium.

Myeloma cells are mixed and fused with B-lymphocytes.

The products of this fusion are grown in a selective medium. Only those fusion products which are both "immortal" and contain genes from the antibody-producing cells survive. These are called "hybridomas." Hybridomas are cloned and the resulting cells are screened for antibody production. Those few cells that produce the antibodies being sought are grown in large quantities for production of monoclonal antibodies (MAbs).

SOURCE: Office of Technology Assessment, *Commercial Biotechnology: An International Analysis*, 1984. Adapted from Y. Baskin, "In Search of the Magic Bullet," *Technology Review*, October 1982.

Lymph nodes or spleens of immunized animals are then removed, and their cells are dispersed and added to cultures of certain types of mouse myeloma cells. The mixed cells are incubated so that their cell membranes are allowed to fuse together, producing hybrid cells with two nuclei. After a period, the hybrid cell nuclei stabilize, and the cultures, consisting of thousands of cells, are placed into a medium in which the growth of the original myeloma cell line is prevented. Only the hybrid cells are now capable of proliferation in the new culture conditions, and the task at this point is to identify and separate those cells that are active secretors of a desired antibody. This is accomplished through any of several types of screening assay, in which the overlying culture medium is tested for the presence of antibody by incubation with a particular antigen labeled (with a radioactive or fluorescent marker) to permit the ready identification of resultant antigen-antibody complexes. Those cell populations demonstrating antibody production are then diluted, and each surviving hybridoma cell is isolated to start a clone of genetically identical cells. The result of this process is the development of large colonies, each derived from a single hybrid cell, and each secreting one specific—hence "monoclonal"—antibody.

Milstein has written (223):

> A monoclonal antibody is a well-defined chemical reagent that can be reproduced at will, in contrast to a conventional antiserum, which is a variable mixture of reagents and can never be reproduced once the original supply is exhausted.

All progeny cells from a single hybridoma clone secrete antibody molecules whose variable regions are identical—the variation is only between, not within, clones.

A single experiment may yield a large number of hybridoma clones, about 10 percent of which secrete a monomolecular antibody specific to that particular cell line. Some of these may be of little or no interest to the investigator, who now faces the lengthy and arduous task of determining the specificity of each secreting hybridoma clone and deciding on its relevance. In some experiments, it may be the goal to generate a variety of different hybridomas. More commonly, an investigator wants to obtain a highly specific antibody. Therefore, he or she attempts to inoculate a correspondingly purified antigen into the B-lymphocyte-contributing mouse in order to reduce the "noise" of unwanted hybridomas.

Hybridoma-derived clones of secreting cells may be propagated in culture media, from which the specific antibody may be separated. Since these cells are derived in part from mouse myelomas, they will induce tumors in mice, with the production of relatively large volumes of antibody-containing ascites fluid. This fluid can be harvested for use, and the hybridoma line can be perpetuated by transfer from mouse to mouse.

A particularly intriguing application of MAbs is in the production of what are referred to as "anti-idiotypic" antibodies. In this technique, animals are immunized with a previously prepared MAb made against a disease-causing organism. The idea is to stimulate the production of a complementary "anti-antibody" which may substitute for the antigen against which the original monoclonal was raised. In an early application of this method to a tropical disease pathogen, Sacks and Sher (303) found that several specific anti-idiotypic antibodies were able to protect mice against challenge with *Trypanosoma rhodesiense*, while others were ineffective. Other investigators have recently reported successful immunization of mice against the bacterium *Streptococcus pneumoniae* with an anti-idiotype vaccine (22). The technique is certain to have wider application, although its eventual usefulness is unknown.

Applications of Genetic Tools to Tropical Diseases

Applications of genetic tools to tropical medicine have been discussed extensively by Falkow (112) and Cross (81), who indicated some uses and advantages of cloned genes in medical and veterinary science:

- elucidation of genetic basis of normal and abnormal cell function;
- diagnosis of disease and disorders;
- synthesis of biologically active proteins; and
- production of subunit vaccines, which may provide:
  - —improvements in existing vaccines,
  - —production of new vaccines,
  - —reduction in adverse side effects as a result of increased purity,
  - —safety in manufacture and use, and
  - —consistency in manufacture and use.

The many applications of MAbs to parasitic tropical diseases have been discussed by Mitchell and Cruise (237), Mitchell (235,236), McBride (218), and Rowe (295). In brief, the uses of MAbs in tropical diseases caused by parasites are:

- for diagnosis of infection and identification of the particular type or strain of parasite,
- for information about the genetics and relationships among the organisms,
- as "probes" for defining the antigenic composition of the parasites and of their products,
- for identification of the parasite components likely to stimulate host protective responses, with possible vaccine production,
- for standardization of diagnostic reagents and vaccines,
- possibly for drug targeting or other means of immune-based treatment of infected hosts, and
- to effect host protection in vivo.

MAbs can also stimulate production of anti-idiotypic antibodies and are widely used as probes and reagents in molecular biology laboratories to detect expression of cloned parasite DNA by host cells.

In the area of tropical medicine, the genetic tools find their greatest potential application in the search for protective vaccines. If a parasitic organism contains a particular antigenic portion that can stimulate protective immunity in humans, it would be desirable to obtain large amounts of that specific antigen (isolated from the remainder of the organism) to use as a vaccine. One way to obtain such material might be to grow the parasite either in animal hosts or in laboratory cultures, and then extract the desired antigen by chemical means. Because of the time and expense involved, this approach is feasible only for experimental work in the laboratory. However, identification, isolation, replication, and production of the antigen by the expression of the DNA sequence responsible for antigen production could provide access to an essentially unlimited and relatively inexpensive source of the immunizing material.

Biotechnology research with agents and vectors of tropical diseases requires a laboratory equipped with a wide range of modern apparatus, including optical and photographic equipment, gel electrophoresis devices and power supplies, centrifuges, refrigerators and freezers, sterilization facilities for culture media, biological safety cabinets, and myriad smaller devices. A great number of chemicals and reagents are also necessary. Facilities for growing the disease organism must be maintained or the organism must be obtained from elsewhere. Trained technical personnel are indispensable. The work is demanding of effort, skill, and imagination, and requires a certain level of financial support. Most developing countries do not have the capacity to do this type of research, so nearly all of it is done in the United States and other developed countries.

6.
Vector Control Technologies: Selected Tropical Diseases

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6.

Vector Control Technologies: Selected Tropical Diseases

INTRODUCTION

The discovery at the turn of the century that some tropical diseases were transmitted by mosquitoes was a major breakthrough in understanding these diseases. The control of mosquito vectors was the earliest and often the only method of intervention against many tropical diseases. Early public health workers, such as Gorgas in Cuba and Panama, and Watson in Malaya, were able to use vector control methods such as drainage and filling, or larviciding standing water with oil and the dye "Paris green," with spectacular success in controlling the carriers of malaria and yellow fever. These methods continued to be used until the advent of the DDT era in the mid-1940s.

This chapter reviews the current status of control technologies for the insect and other arthropod vectors of diseases such as malaria, filariasis, leishmaniasis, and arboviral infections (e.g., yellow fever). It also reviews the status of control technologies for certain mollusks (e.g., snails) that are essential in the life cycles of trematode parasites that cause diseases such as schistosomiasis. The terms vector biology and control have traditionally applied only to insects and other arthropods that carry diseases. Currently, however, these terms also apply to snail intermediate hosts.

Control of vector-borne diseases is possible only through detailed and accurate understanding of the factors involved in transmission. Such understanding is gained through meticulous and tedious observation and experimentation wherever the disease exists. Understanding the role of the vector necessarily overlaps with field studies of the human host's interaction with the environment. Cultural practices, such as storing barrels of drinking water without lids (which provide perfect breeding sites for mosquitoes), or poor sanitary habits need to be studied to best design social intervention techniques.

Prospects for the future, including the relatively new concept of integrated pest management (IPM), are summarized in this chapter. Vector bionomics, the study of vectors and their interactions with the environment, provides the scientific rationale for interventions against vectors and is described briefly below.

VECTOR BIONOMICS

Progress in vector research tends to come in small increments that relate to better understanding of vector bionomics (the study of vectors' habits and their interactions with the environment). Such research is often narrowly focused research, from which extrapolations can only infrequently be made to other vector control situations. Studies of mating habits, host preferences, oviposition (egg laying) behavior, larval habitats, flight densities, and natural infection rates with pathogenic organisms provide insights that add to our ability to understand transmission dynamics and to target interventions against susceptible vector species.

Information about the resting habits of mosquito vectors of malaria has provided the rationale for, and accounted for the success of, the indoor residual spraying of insecticides in many tropical areas. Because some mosquitoes rest on a surface before and after the blood meal, often near the person they have bitten, spraying all available resting surfaces (the walls, ceilings, undersides of furniture, etc.) with a long-lasting insecticide (e.g., DDT) has been the method of attacking the vector species.

In many cases, this approach to malaria control has led to great reductions of malaria prevalence. (In some areas of antimalarial spraying, sandflies, vectors of leishmaniasis, have also been controlled.) Unfortunately, in many cases, lack of information about the behavior of specific populations of mosquitoes has led to failure. Some species, or subpopulations within species, exhibit a behavior pattern of entering a house to feed, but resting outside, or actually avoiding sprayed surfaces. For example, the malaria vector *Anopheles nuneztovari* in parts of Colombia and Venezuela enters houses, obtains a blood meal, and then leaves immediately to rest outside, without acquiring a lethal dose of DDT (107). *A. minimus flavirostris*, an important vector of *Wuchereria bancrofti* (filariasis) in Luzon and Palawan, the Philippines, enters houses to feed on humans only during the middle of night, when the microfilarial stage of the parasite is at its highest concentration in the blood. This particular mosquito also leaves the house to rest outside, and is unaffected by indoor spraying of DDT.

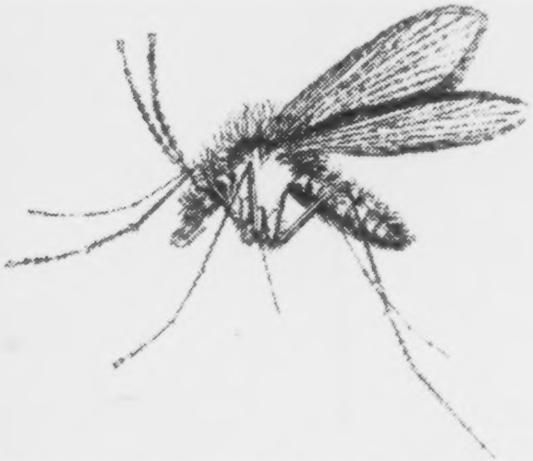

Photo credit: H. W. Brown and F. A. Neva, Basic Clinical Parasitology (East Norwalk, CT: Appleton-Century Crofts, 1983). Reprinted by permission.

Sandfly, *Phlebotomus*, vector of leishmaniasis.

Control of the primary vector of a tropical disease does not always result in successful control of the disease. The disease may continue to be prevalent and a second previously unsuspected vector may be discovered. In one case, the elimination of stagnant groundwater as a breeding site for malarial vectors was followed by the discovery of another vector breeding in tiny pools of water trapped in the fronds of palm trees.

Work in the field of vector bionomics is progressing at a productive, though undramatic, pace. This area is grossly understaffed and underfunded, and there are critically few new scientists entering the field (253). Some progress of a fundamental nature has occurred in the laboratory. For example, recent work has provided information about the importance of mosquito saliva, which functions as an anticoagulant, in the mosquito's proboscis (hollow tongue). The mosquito's saliva promotes the formation of a blood gradient in tissues as it probes, helping the insect to find a blood-filled capillary. Malarial sporozoites in the mosquito damage the mosquito's salivary glands, reducing the production of saliva; the reduced production of saliva, in turn, causes an increase in probing time, and thus promotes greater transfer of sporozoites to humans being bitten (329).

Research in both the field and laboratory aspects of vector bionomics is time- and labor-intensive, but is a necessary activity for the eventual control of vectors. As noted in a study of pest control by the National Research Council (253):

> The control of arthropod vectors of disease or other pests of public health should be attempted only with recognition, and insofar as possible, an intimate knowledge, of the significance of the ecology and behavior of the target species and the epidemiology of the disease and with appreciation for environmental values that may be depreciated.

CONTROL OF ARTHROPOD VECTORS

Insecticides

Insecticides, classified as either chemical or biological, remain the primary vector control intervention. Two strategies are involved in the use of insecticides: 1) reducing the population size of the target vector (e.g., by treatment of a breeding site to kill immature forms); and 2) reducing the longevity of adult forms of the vector, so that they die before transmitting the disease (e.g., by residual house-spraying to intercept only those adult forms that feed on humans). Regardless of the strategy, all insecticides act as a selective mechanism against disease vectors, resulting in the development of resistant insect populations that have lost their susceptibility to the particular agent in use.

Chemical Insecticides

Chemical insecticides began to dominate the field of vector control around the end of World War II, with the widespread use of DDT. It was at that time that the World Health Organization's (WHO) worldwide "malaria eradication campaign" began, and through that experience that the world began to learn about the amazing capacity of insect populations to evolve that are resistant to pesticides (see box 6-A). The development of resistance called for using even more lethal chemicals.

Most insecticide development today is directed at pests of agricultural importance rather than at medically important vectors of diseases. Although many new chemical insecticides are produced and tested annually, these new chemicals are not necessarily appropriate for specific disease vectors. Also, because of research and development costs, new chemicals are expensive. The development of insects that are resistant to new chemical insecticides is a virtual *certainty*. The only question is simply how quickly the emergence of resistance will cause the insecticide to be abandoned.

In spite of the gloomy prospects for the complete success of chemical pesticides, such pesticides still form the backbone of vector control programs and are responsible for controlling disease in many areas. Even DDT, which has been banned for agricultural use in the United States and many other countries, is still a useful chemical.

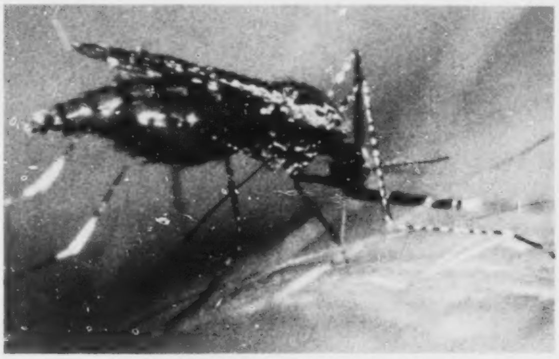

Photo credit: Robert Edelman, National Institutes of Health

An anopheline mosquito, vector of malaria, after a blood meal.

DDT was not banned for public health use (which is far more circumscribed than agricultural use), however, and it continues to be used in many countries worldwide for residual house-spraying against vectors, especially those of malaria. The only constraint on its use is resistance in local vector species.

The emergence of DDT-resistant strains of mosquitoes and the need to decrease the use of DDT have spurred the development of new insecticides. The principal compounds that have been substituted for DDT are malathion, fenitrothion, and propoxur. Another insecticide, pirimiphos methyl, has been field tested as a substitute for malathion in several sites where local mosquitoes have developed malathion tolerance (e.g., in Turkey and in Pakistan).

All of the newer chemical insecticides have greater immediate toxicity to humans and animals than DDT has. The irony of the replacement of DDT by other, more quickly degrading chemicals is that in order to be effective within a limited time span, the new chemicals must also be highly toxic. Thus, although the long-term environmental effects of DDT's persistence have presumably been reduced, the short-term hazards to users (i.e., workers in-

Box 6-A.—The Malaria Eradication Campaign

In most of the world, malaria has not been eradicated or even controlled. According to the World Health Organization (WHO), the hundreds of millions of people living in nearly all of the "once malarious" areas of the world are still at risk. This situation exists, despite the initiation by WHO in 1956 of a "Malaria Eradication Campaign." The story between then and now is one of transient gains against the mosquito vectors and *Plasmodium* parasites, followed by recrudescence approaching pre-eradication levels. An obvious lesson from the malaria eradication effort is the imperative for an approach to control that integrates strategies for reducing disease transmission and plans equally well for both active control measures and maintenance of gains. The practical application of knowledge about parasite and vector biology is increasingly important as well.

Although antimalaria drugs played a supporting role in the campaign, the hero was to be DDT (dichloro-diphenyl-trichloro-ethane). Initially, the eradication of the *Anopheles* mosquito vectors of malaria was the goal, though the necessity for eradication vanished when it was discovered that stopping transmission of malaria for about 3 years, and maintaining active surveillance for introduced cases, could eliminate the disease permanently.

The war on malaria was begun after initial successes in the 1940s, at the time DDT became available. But by the time WHO organized behind the eradication campaign, signs of the program's undoing were already present. Mosquitoes resistant to DDT began to appear in the second year of the program. Nonetheless, the program went ahead. Fifty-two countries around the world launched eradication programs. Ten of those countries achieved their goal and have retained their achievement. None represents major tropical areas.

India and Sri Lanka achieved the greatest successes in eradication, so the resurgence of malaria in those countries represented the most visible and disappointing failures. India had reduced the number of annual malaria cases from an estimated 75 million in 1951 to about 50,000 by 1961. In Sri Lanka, 29 cases were reported in 1964, down from about 3 million cases in the years after World War II. From India's 1961 low, incidence rose steadily, with over 1 million cases again in 1971. At that point, mosquito spraying was resumed, only to fail because of insect resistance and rising insecticide prices. By 1977, India had an estimated 30 million to 50 million cases of malaria.

Successful malaria eradication, using the precepts of the WHO program, would have required not only the technology of DDT, on which all hopes were pinned, but to an even greater extent, the political and social will to carry out programs. Spraying of dwellings at regular prescribed time intervals and constant surveillance in later phases were fundamental, but largely unachievable worldwide. The area of greatest malaria endemicity, Africa, was not included in the eradication campaign for both technical and political reasons.

Officially, it was recognized that there would be only one chance to succeed at malaria eradication. As initially planned, the active eradication efforts with massive spray programs would last 3 or 4 years. Problems in implementation strung out efforts over 10 or more years in many countries. By that time, resistance to DDT and other insecticides was guaranteed to call a halt to progress. What was not accomplished in 3 years had ever smaller prospects for success as the years wore on. Added to the technical problems was the fact that during periods of social and political instability in parts of Latin America, Africa, and the Middle East, malaria eradication was accorded a low priority.

This somewhat downbeat story is not hopeless, however. Eradication of malaria still is possible in some areas. Cuba achieved eradication in 1973. Most of Venezuela and Argentina and much of Brazil and Paraguay are free of malaria.

There is mixed emotion and opinion in judging the malaria eradication program. According to Harrison (150), many in WHO feel that it was successful overall. Harrison asserts, however (150):

> . . . [the program] had one unqualifiedly negative result: It all but destroyed malariology.

> **It turned a subtle and vital science dedicated to understanding and managing a complicated natural system—mosquitoes, malarial parasites and people—into a spraygun war.**

The goal of malaria eradication has now been laid aside, and current efforts to control the disease have not even held the advances made during the program. There is today an urgent need for the development of new, rational control strategies for malaria.

volved in the manufacture and application process, and people and wildlife inhabiting the areas being sprayed) have probably increased. The new insecticides are also many times more expensive than DDT, a problem that has greatly limited their use in the endemic countries unable to afford them.

Biological Insecticides

Various agents called biological control measures (see below) are in many cases more accurately described as biological insecticides. The biological insecticides include pathogenic bacteria, fungi and their products, and growth hormones. In most cases, the function and application of biological insecticides are completely analogous to those of synthetically manufactured insecticides. Often, the two types of insecticides differ only in that the former are produced by a micro-organism and the latter are produced by chemical synthesis. Most biological insecticides are directed against larval forms of arthropods, which means they are suitable only where conventional larviciding would also be feasible (e.g., where the breeding sites are easily found and treated).

Despite the appeal of using naturally occurring agents, it is not safe to assume that biological insecticides will be nonpathogenic to humans, nor that because these agents occur naturally, the target organism is less likely to develop early resistance.

Pathogenic Bacteria.—The cultivation and application to vectors of infectious or toxic agents such as viruses, bacteria, fungi, and helminths is an area of active research. So far, however, there is only one practical application in use, *Bacillus thuringiensis israeliensis* (BTI), of which the most common strain is called *B.t.* H-14.

BTI was first isolated in Israel in 1976, and extensive research has revealed that the bacterium produces a toxic crystalline compound (delta-toxin) fatal to virtually all insects when they ingest it. Now commercially produced, this toxin is used on farms and gardens, as well as in public health projects, in the United States and around the world. *B.t.* H-14 is now applied in the control of the *Simulium* (blackfly) vectors of onchocerciasis in West Africa. A number of field trials using *B.t.* H-14 against mosquito species are in progress.

BTI differs in its mode of action from other insecticides. It is not readily susceptible to enzymatic degradation or other biological processing within the insect. Although there is no reason to believe that BTI will differ from other insecticides in placing selective pressure on heavily exposed vector species, resulting in the eventual emergence of resistant strains, development of resistance is not expected to emerge quickly.

Following from the success of *B.t.* H-14, the search for other pathogenic bacteria has intensified. Several promising strains of *Bacillus sphaericus* have been isolated and are undergoing evaluation as larvicides.

Although biological insecticides are often assumed to have few, if any, adverse effects on humans, a recent report (305) described a corneal ulcer in a farmer following splashing of the eye with a biological agent. BTI was subsequently isolated from the ulcer. This may be an extremely rare occurrence or early warning of a potential problem.

Fungi.—Several species of fungi are being applied to vectors of disease in the hope of producing a lethal fungal infection. Those in the development and field testing stage include *Tolypocladium cylindrosporum*, *Culicinomyces clavor-*

sporus, and *Coelomomyces* spp. An earlier promising agent, *Metarhizium anisopliae*, has been found ineffective in the field.

Growth Hormones.—The identification and testing of insect hormones that alter growth and reproductive behavior is another area of research. The most notable example is juvenile hormone, an essential and naturally produced mediator of the growth process, which regulates insect development during pre-adult stages.

When applied in excess to insect breeding sites, juvenile hormone can prevent maturation of larvae and thus function as a biological larvicide. Safety and efficacy arguments regarding the use of this hormone as a larvicide are similar to those surrounding BTI. A drawback to the use of juvenile hormone is that resistance has now been demonstrated in some species, contrary to all earlier expectations. The high cost of juvenile hormone has limited its use in public health projects. Furthermore, juvenile hormone is useful only where larviciding is a feasible intervention.

Biological Control Measures

To control arthropod vectors of tropical diseases, various ingenious genetic and biological control procedures have been attempted. Many are based on the concept of introducing either sterile males or predators to a vector population, or by breeding arthropods that are resistant to infection by disease pathogens.

Introduction of Sterile Males

Large numbers of sterile hybrid males or of males sterilized by irradiation or chemicals can be released into the vector population. To the extent that sterile males outcompete normal males for females, the vector population is reduced.

The release of sterile males to control screwworm flies has been a spectacular veterinary success. Unfortunately, however, methods involving the release of sterile males are not practical for the control of mosquito vectors, nor indeed for most other medically important arthropods. Tsetse flies or other vectors with low density or reproductive potential may be controlled in this way.

Introduction of Predators and Competitors

The introduction of predators or competitors in vector populations has been tested a number of times, with variable results. These methods cannot be used in a broad-brush manner because of the many variations in the ecology and epidemiology of vector-borne disease. Only vectors breeding or existing in habitats suitable to the predator are susceptible to these methods.

The efficacy of using larvae-eating fish, such as *Gambusia* spp. and *Aplocheilus* spp., to control mosquitoes that transmit malaria is under study in a number of trials. This method works well against vectors that breed in large ponds or rice paddies, and a successful trial in northern Somalia is being extended (353).

A different tactic involves the use of cannibalistic larvae of the mosquito genus *Taeneorhynchus*. These larvae eat other larvae present in their breeding waters. The method is limited, however, to those species which share the same breeding habits as the predator.

The use of nematodes, such as *Romanomermis culicivorax* and *R. iyengari*, is under study. These worms prey on the immature forms of some insects.

Detection of Species Complexes

Scientists are investigating improved arthropod vector control using powerful new methods available for assessing genetic differences within vector species complexes. The elucidation of species complexes allows seemingly identical species to be differentiated according to their capacity as disease vectors. Once differentiated, highly specific field interventions may be initiated against the most capable vector species.

The concept of a species is theoretically simple, centering on reproductive isolation. In the case of large plants or animals, morphological and behavioral differences can be fairly easily observed and correlated to infer uniqueness as a species. In the case of many vector species, however, visually identical specimens may exhibit diametrically opposed behavioral habits (e.g., visually identical specimens of mosquitoes appear to breed in both freshwater and saltwater).

Past questions of species identity were frequently resolved through analysis of all stages of the life cycle (egg, larvae, pupae, adult) using classical taxonomic procedures, which were labor- and talent-intensive. Early attempts sorted out conflicting behavior patterns by tedious cross-breeding experiments which demonstrated reproductively incompatible populations (populations whose matings produced either no or sterile progeny).

Several alternative technologies are now available which, in conjunction with classical taxonomic and ecologic studies, can answer questions of vector identity. Cytogenetic studies may be performed using photomicrographs of the chromosomes in the salivary glands of insects. (Chromosomes in the salivary glands exist as multiple intertwined copies which are large enough for isolation, preparation, staining, and photomicrography, allowing for mapping and comparison of banding patterns of the genes.) In addition, isoenzyme electrophoresis has been used to investigate questions of insect species and strain similarity. Certain enzymes, common to all related species, are functionally identical but differ in their chemical composition. The differences can be detected and used to identify separate species or strains. This method is being assessed on wild-caught mosquitoes in Tanzania (353). More recently, DNA hybridization techniques have been developed to demonstrate whether or not a match exists between the chromosomes of insects whose species identity is uncertain (94).

Identification of Disease Organisms Within Vectors

In addition to problems of controlling insect vectors, there are difficulties in determining whether and to what extent a vector is harboring a disease-producing organism.

Immunologic work on malarial sporozoite vaccines (see ch. 7 and case study B) has resulted in the development of two methods to detect sporozoite forms of the malaria parasite in mosquitoes. Each species of the malaria parasite (*Plasmodium* spp.) has one predominant, unique surface antigen, and monoclonal antibodies (MAbs) against each of the different species' antigens have been produced. Using these MAbs, investigators developed a radioimmunoassay (RIA) and an enzyme-linked immunosorbent assay (ELISA) (36,435) that can sensitively and specifically identify the presence of sporozoite forms of the malaria parasite in crushed preparations of field-captured mosquitoes (69). These immunoassays replace the previous method of detecting sporozoites, which was manual dissection of the salivary glands from individual mosquitoes, followed by microscopic examination. (See ch. 8 for a full discussion of these diagnostic tests.)

The RIA and the ELISA greatly facilitate several types of field investigations and may lead to better understanding of malaria transmission dynamics and vector control. They will allow field researchers to: 1) identify new vector species; 2) determine the relative prevalence of the various malaria species, specifically those extremely uncommon or clinically or microscopically unrecognized; 3) correlate vector species with transmission of particular malaria species; and 4) quantitate each vector species' role in transmission (vectorial capacity) by its infection rate.

Similar technologies will probably be developed for the diagnosis of other vector-borne pathogens, and this will greatly facilitate field research and surveillance of diseases such as onchocerciasis and filariasis (155).

Control of Trypanosomiasis Vectors

Two insect vectors against which some progress has been made are the tsetse fly, conveyor of African sleeping sickness (African trypanosomiasis, and the reduviid (or kissing) bug, which spreads Chagas' disease (American trypanosomiasis).

Tsetse Fly Control

Field research on the life cycle and bionomics of tsetse flies (*Glossina* spp.) has demonstrated differential susceptibility and vectorial capacity among fly populations. Various techniques of tsetse fly control have been used over the decades, including the placement of sticky patches on the backs of plantation workers, clearing of fly habitat vegetation along river banks, and ground and air spraying of DDT and other insecticides. As yet, no tsetse fly resistance to insecticides exists, and in fact, the insecticides are highly effective, though expensive.

Field trials of tsetse fly traps are under way in various locations. A trial in Burkina Faso (formerly Upper Volta) achieved excellent success in reduction of *Glossina* numbers using a biconical trap (195,353). A cheaper monoconical trap has also been developed, which is insecticide-impregnated and attracts flies by odor. Insecticide-impregnated screens and car tires have also been tested (353).

Reduviid Bug Control

Currently, control of the reduviid bug vector of Chagas' disease is the only practical measure against this disease. Over the long term, improvement of rural housing is the single most important intervention against Chagas' disease. Poorly constructed, substandard housing provides both breeding and resting sites for the reduviid bugs. Periodic spraying of residences with residual insecticides is one method of control. A recent innovation has been the application of paints and plasters containing slow-release malathion, which controlled the vector for up to 1 year.

Photo credit: Dr. Robert Edelman, National Institutes of Health

Reduviid bugs, the vectors of Chagas' disease, live in the thatched roofs of houses such as this one.

CONTROL OF SNAIL INTERMEDIATE HOSTS

Although mosquitoes, biting flies, and other blood-feeding insects are the usual emphasis of vector control programs, one of the most important tropical diseases, schistosomiasis, involves snails in the transmission cycle. The snail is actually an intermediate host rather than a true vector, as the parasite is released by the snail into a body of water and enters through the skin of a human host when the person comes in contact with it through bathing or wading.

Controlling snail populations, like controlling insect vectors of diseases like malaria, is one way of controlling the spread of schistosomiasis. Snail control methods are analogous to those used in insect control, namely alteration of snail habitats and molluscicding (direct poisoning of snails).

Artificial lakes and irrigation projects can lead to the spread of schistosomiasis, through the expansion of habitats for the snail intermediate host (390). Various environmental or engineering measures have been used successfully for snail control, such as irrigation canals lined with cement in Japan, and reclamation of swamps in the Philippines. Molluscicidіng was one of the interventions used to reduce the incidence of schistosomiasis on the island of St. Lucia (see ch. 5). Each approach is potentially effective, but requires careful management and is expensive.

Molluscicides

Attempts have been made to control snail hosts since the early 1900s, when the role of snails in the schistosomiasis transmission cycle was elucidated. A number of chemical compounds have been used over the decades. Independent of the agent used, an important concept in snail control has been the use of "focal molluscicding" which concentrates effort on the often localized areas of actual transmission (58,284). Since only those areas of known transmission are molluscicided, focal molluscicding saves chemicals and personnel. However, good diagnostics and surveillance mechanisms are needed for focal mollusciciding to be effective. For molluscicides, as for insecticides, both biological and chemical agents have been used.

Chemical Molluscicides

Two molluscicides are commercially available: copper sulfate, available since 1920, and niclosamide, available since 1959. Scientists have recently investigated slow-release compounds, which would reduce the logistical demands of frequent, periodic applications to snail habitats (176). Organotin compounds, very cheap and shown to be highly effective in laboratory and field trials, are undergoing research and development (176).

The development of chemical means of controlling snails has been hindered by a lack of information about the biochemistry and metabolism of the snail intermediate hosts and about the mode of action of molluscicides. B-2 (a dichlorobromophenol) is a molluscicide used extensively in Japan (353). It has been shown to affect several important glycolytic metabolic steps in *Oncomelania* and *Biomphalaria*, two important genera of schistosomiasis vectors.

Plant-Derived Molluscicides

Plant-derived molluscicides have been investigated as possible alternatives to chemicals for a number of years, because plant-derived compounds could be less costly and would probably be less toxic to forms of life other than snails. The assumption is that plant-derived compounds will pose less of an environmental hazard than chemicals, and it is possible they could be developed through village level, self-help schemes (399).

Screening for potential molluscicides has concentrated on plants with known medicinal properties. Many of these have been identified as containing active molluscicidal compounds. The most thoroughly studied is *Phytolacca dodecandra*, whose berry extract (endod) has been used in Ethiopia. Other promising species are *Ambrosia maritima* and other species of that genus, and *Anacardium occidentalis*.

The search for effective plant-derived molluscicides has been impeded by the lack of knowledge about snail metabolism and physiology. Currently, the Special Program for Research and Training in Tropical Diseases (TDR) is promoting the development of screening methodology and developing goals for the use of plants (353).

Biological Control Measures

The most promising biological agents for controlling the snails involved in the transmission of schistosomiasis appear to be competitor snails, such as *Marisa cornuarietis*, which has been used in Puerto Rico. In the course of browsing over vegetation, *M. cornuarietis* eats all stages of the snail *Biomphalaria* spp. Other competitor snails under study are *Pomacea*, *Thiara*, and *Helisoma* spp. (176,353).

Detection of Species Complexes

As is true in the case of many insects, susceptibility to infection in the case of snails varies between species and strains. Isoenzyme electrophoresis has been developed for the detection of species complexes in various snail populations (173,174) and used experimentally to identify snail populations with differing susceptibility to schistosome infection (227). Knowledge of susceptibility will increase our understanding of the epidemiology of schistosomiasis transmission.

INTEGRATED PEST MANAGEMENT (IPM)

A new emphasis is being placed on an integrated approach to vector control based on IPM strategies. IPM strategies emphasize the need for combining basic field studies of vector bionomics—the study of the interaction of vectors with their environment—with biological and chemical control agents. In contrast to single-tactic programs based on chemical insecticides, IPM stresses multifaceted environmental measures reduce disease transmission. In the Panama Canal Zone, an

IPM-type program eliminated malaria; then DDT was added to the control program, and screening and drainage practices were neglected. The single-tactic approach failed when the mosquitoes became resistant to DDT, and malaria returned.

Theoretically, IPM requires an intimate knowledge of all factors relating to vector biology and disease transmission. Critical factors are selected and monitored for change, and tailored intervention techniques are applied as needed to control the vector. Successful IPM models are based on the analysis of factors such as weather/climate, vector density, pathogen infection rate, and other indicators that are of predictive importance for disease transmission. In practice, IPM has sometimes meant nothing more than using several insecticides in combination, instead of just one.

Paradoxically, in many vector control projects, intuitive intervention may lead to counterproductive results (329). For example, insecticide spraying may reduce the population of larvae in a breeding site, but have the unexpected result of producing more robust adults, better able to transmit disease, because of reduced competition for food.

RESEARCH NEEDS

Insecticides and Molluscicides

The search for cheap, safe, and effective insecticides and molluscicides, whether of chemical or biological derivation, characterizes research in all vector-borne diseases. The lack of an obvious profit element greatly inhibits commercial development of any insecticide having *only* public health applications. However, agricultural pests remain a subject of intense research, and there will quite likely be benefits for public health problems.

Two methods are used in developing new insecticides. In the first case, knowledge about toxic substances is used to test known and newly synthesized compounds against a range of target species. In the second case, knowledge is developed about the physiology and biochemistry of target species in order to identify critical enzyme pathways or other life functions against which insecticides could be made. Further research is needed in either instance.

Vector Bionomic Studies

There is a continuing need for better understanding of the biology of all disease vectors: identification of new vectors, vectorial capacity, physiology, genetics, insecticide susceptibility, behavioral characteristics such as biting, resting, and breeding habits, and any other factors that contribute to the maintenance and transmission of disease.

Improved means of detecting disease-causing agents within vectors is an important area in need of research and development. There is a vital need for increased numbers of trained personnel at all levels of research (253).

SUMMARY

Attempts to control arthropod vectors and intermediate hosts such as snails are relatively recent, proceeding from the discovery of their role in human disease transmission around the turn of this century. For several decades, physical methods were the only means available for controlling vectors of tropical diseases. Study of the behavior and natural history of vector species was encouraged by the requirements of physical control methods. After World War II, the old methods were largely abandoned in favor of synthetic chemical insecticides, whose promise was to eradicate vector species, particularly the mosquito vectors of malaria.

The field of vector control is slowly emerging from total reliance on chemical control methods toward IPM (integrated pest management). IPM, at least theoretically, includes chemical, biological, and physical control methods, once again requiring more intimate knowledge of the lives of vectors, now including knowledge of their genetic and biochemical characteristics. IPM is as yet a new field, but one in which there is a great potential to influence the occurrence of disease in developing countries.

It is unlikely that any new medical intervention, whether a chemotherapeutic drug or a vaccine, will be sufficient by itself to reduce significantly the prevalence of vector-borne tropical diseases. To control vector-borne tropical diseases, several methods adapted to local conditions are probably necessary.

Public health authorities are beginning to emphasize basic vector control engineering measures, such as drainage, filling, and control of water bodies. However, these physical measures alone are not sufficient for disease control, and unless new methods are developed, there will be few if any practical alternatives to insecticides.

7.

Immunization Technologies: Selected Tropical Diseases

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7.
Immunization Technologies: Selected Tropical Diseases

INTRODUCTION

Vaccines exist for many of the viral and bacterial diseases that are important in developing countries, but there are no vaccines against the parasitic diseases that are generally associated with the tropics. Antiparasite vaccines are more difficult to formulate than vaccines for viruses and bacteria, the reason being that protozoan and helminthic parasites are more complex in biochemical and physical composition, life cycle stages, and interactions with their hosts. The immunology of parasitic diseases is in a vigorous phase of research (66,164,213,241,273) and forms the underpinning for attempts at vaccine development.

The available vaccines vary in the protection against disease that they induce and the risks of adverse effects in individuals who are vaccinated. Vaccines against yellow fever, measles, rubella, and mumps, for example, are almost always effective and provide for long-term protection. Others—influenza, pneumococcal pneumonia, cholera, and typhoid, for instance—do not induce immunity in as high a proportion of those vaccinated and may last only a matter of months or a few years. Both new vaccines against diseases currently not immunizable and improvements in existing vaccines are needed.

There is great optimism in vaccine research today. Large strides in biotechnology, particularly in the use of monoclonal antibodies (MAbs) and recombinant DNA, and a rapidly growing body of knowledge in immunology are aiding the field of immunization technology. The development of replacement vaccines for viral and bacterial diseases that are safer, more effective, and possibly less expensive than vaccines currently available, and the development of new vaccines against some tropical diseases, including parasitic diseases for which no vaccines now exist, are advances that now depend more on research with the tools already at hand than on major technological breakthroughs (310).

The single most visible, exciting development in vaccines today is that a vaccine against malaria may be ready for widespread use in a matter of a few years. In 1984, progress could be followed almost weekly, as the immunologic properties of various life-stages of malaria parasites were characterized in laboratories in different parts of the world and the information was processed for practical significance to a vaccine.

IMMUNITY AND VACCINES: BACKGROUND

Immunity

The term "immunity" refers to the capacity of an organism to resist a particular disease. Disease-causing organisms themselves require certain conditions for survival, and only certain potential host organisms will provide those conditions. Thus, as a species, humans are susceptible to a unique set of pathogens that differs from the set of pathogens that cause disease in other species, although there are areas of overlap (zoonoses). "Species immunity" does not stem from a response of the potential host's immune system against the disease, but simply from an inability of the disease organism to become established. From an evolutionary point of view, such immunity is important, but from the standpoint of preventing diseases and developing vaccines against them, the important kind of immunity is that which individuals have or can develop against diseases that do occur in human beings.

The immune status of an individual is built from a complex of mechanisms, some genetically determined and some acquired during life, which we are just beginning to understand. A person with complete immunity will not get a disease when exposed to the causative agent. The converse is complete susceptibility, which means that anytime the person comes in contact with the causative organism, the disease will develop. Between solid resistance and complete susceptibility, a range of states of partial immunity and partial susceptibility exist.

"Innate immunity" is genetically determined at birth. Many people of African descent are resistant to *Plasmodium vivax* malaria because they lack a protein on their red blood cell surface that normally allows the malaria parasite to bind to the red blood cell. Many people in endemic malaria regions are resistant to *P. falciparum* malaria because they carry the sickle-cell or thalassemia trait. Genetically determined aberrant types of red blood cells cause sickle-cell anemia in individuals who inherit the aberrant trait from both parents (homozygotes), but prevent malarial infection in individuals who have inherited the trait from only one parent (heterozygotes).

"Acquired immunity" is immunity that results either from the body's response to exposure to disease or from vaccination ("active immunity") or from the transfer of antibodies from another person or from animals ("passive immunity"). Active and passive immunity are discussed further below.

Active Immunity

"Active immunity" is immunity acquired by an originally susceptible individual by effective contact with an infectious organism (or a closely related species) or its products. Such immunity results when a biochemical entity known as an antigen (which may be on, in, or produced by the infectious organism) stimulates a response by the immune system of the host.

Active immunity is the result of two general types of stimulation of an individual's immune system:

- **humoral immunity**: immunity resulting from the production by antigenically stimulated B-lymphocytes (specialized white blood cells) of nonliving proteins called "antibodies" (or immunoglobulins) that circulate in the blood; and
- **cell-mediated immunity**: immunity resulting from increased activity by living cells in the blood and other tissues (e.g., T-lymphocytes, macrophages/monocytes, eosinophils, mast cells, and natural killer cells) that directly and nonspecifically destroy foreign material in conjunction with antibodies.

Active immunity often is a result of natural exposure to an antigen that results in subclinical or clinical infection (e.g., chickenpox infection), but it may be induced by vaccination (e.g., measles or polio vaccination).

Vaccines usually stimulate humoral immunity, i.e., antibodies against a specific antigen, but some immunizing agents strengthen cell-mediated immunity. An example of the latter is Bacillus Calmette-Guerin (BCG) vaccine, a weakened or attenuated tuberculosis vaccine. Whether stimulated by natural infection or vaccination, active immunity usually takes some weeks to develop and may thereafter be lifelong, as with yellow fever or smallpox immunization, or may wane after a variable period, as with typhoid fever.

Passive Immunity

"Passive immunity" is immunity conferred on an individual by the direct transfer to that individual of antibodies produced by another individual. It may result from direct transfer of immune serum that is obtained from individuals with acquired immunity against a specific infectious agent. Large injections of immune serum globulin against hepatitis ("gamma globulin") are sometimes given to Western travelers to the tropics. Newborns acquire certain antibodies passively through the placenta from their mothers and also receive some protection through antibodies in breast milk. The protection offered by passive immunity is relatively short-lived, usually disappearing within a period of weeks. The transfer of antibodies does nothing to stimulate the body's immune system to produce its own antibodies.

The use of MAbs to confer passive immunity against experimentally attempted infection ("challenge") is important for laboratory investigations and for establishing the theoretical possibility of inducing immunity, but probably will not become a major tool for disease control.

Vaccines

Vaccines are used to produce active immunity. In all cases, a vaccine contains some biochemical compound whose structure is the same as, or similar to, some part or product of the infecting organism. Introducing that foreign material (antigen) stimulates the host's immune system to produce humoral and cell-mediated immunity. Following vaccination, the host's immune system is primed to attack the organism more quickly and efficiently should it ever reinvade the host's body.

Most tropical diseases are chronic infections (e.g., malaria, schistosomiasis, leprosy) in which the proliferation of the pathogen does not exceed a certain level and an equilibrium of sorts is established between pathogen and host. The immune system of the host reacts to the parasite, yet the parasite is not completely eliminated because it has various methods of evading the immune system. The equilibrium reached is "beneficial" to the parasite: the host's body is not overwhelmed or killed by the infection. Parasites evade or subvert the immune system in many different ways. They can lose the surface antigen by which they are recognized by the host, take up host proteins as a surface coat, shed and generate altered surface antigen over time, or internalize in the host's cells. The challenge of current immunology is to make the immune system produce an effective response against the parasite.

A further complexity is that the immune response itself often causes the pathology of the disease. For example, the immune response to schistosome eggs in the host's tissue causes the clinical symptoms of schistosomiasis. In eliciting a protective response by the host's immune system, it is important to avoid stimulating a response that causes immunopathology.

Types of Vaccines

The production and composition of vaccines differ depending on the disease-producing organism and the available knowledge about it. It is no accident that most currently available vaccines are for diseases caused by viruses and bacteria, which have limited variability in their structure, biochemistry, and genetic makeup. Most of these vaccines were created through empirical work, often with very imperfect understanding of the underlying biochemistry and molecular biology.

Conventional Vaccines.—The commonly produced vaccines are derived from actual disease organisms (or closely related organisms) grown in a culture medium, embryonated eggs, or in cell culture. Despite great efforts at quality control, such biological products are always subject to some variation. Potency can vary, improper inactivation may make a virulent vaccine, and organisms may change or mutate in culture, reverting to or developing pathogenicity. Another potential problem is contamination. For example, some early batches of polio vaccine grown on monkey kidney cell cultures were contaminated by a monkey virus. In some vaccines, there may be latent, undetected viruses whose effects may not be evident for years.

The major types of conventional vaccines are the following.

1. **Live infectious organisms:** The infectious agent is obtained from someone who has the disease. For instance, scrapings from lesions of cutaneous leishmaniasis have long been used in endemic areas for deliberate inoculation of children to prevent the possible appearance of disfiguring facial lesions later in life (168).

2. **Closely related organisms that stimulate cross-immunity:** Jenner's discovery in the 18th century that inoculation with cowpox could prevent smallpox marked the beginning of modern vaccination.

3. **Live, attenuated organisms:** Cultured organisms are weakened in various ways so that they

will remain infectious and stimulate immunity but will not cause disease. Polio, measles, rubella, and BCG vaccines are of this type. Among the problems with this approach are the lack of assurance that the attenuation procedures really eliminate pathogenicity while maintaining appropriate antigenicity; the possibility of mutational reversion to virulence; the introduction of pathogen genomic segments into host DNA; and a small but measurable incidence of severe side effects such as encephalitis. The antiparasite vaccines in use in animals are live, attenuated organisms, the most successful of which are the radiation-attenuated bovine lung-worm larvae employed in some countries since 1959 (26). Field trials have also been reported with irradiated larval schistosomes in cattle (338).

4. **Killed (inactivated) organisms:** By heat, formalin, or other methods, the disease-producing organism is inactivated without destroying its immunogenic potential. Typhoid, pertussis, plague, and Rocky Mountain spotted fever vaccines are examples of vaccines produced in this way.

5. **Products of bacteria:** When a specific biochemical product of bacteria produces the disease, this toxin may be altered to a nonpathogenic toxoid, which can be used to immunize. Tetanus and diphtheria vaccines are toxoids.

6. **Parts of organisms (subunit vaccines):** The purified pneumococcal or meningococcal capsule polysaccharide subunit vaccines are prepared by isolating an immunogenic portion of the outer surface of the pneumococcal or meningococcal bacterium. Isolated polysaccharides are only weakly immunogenic, especially in children. Efforts to increase immunogenicity by coupling polysaccharides to carrier proteins are in progress. The term "subunit vaccine" also is used to describe vaccines made from subunits of bacterial toxins (e.g., the subunit-b vaccine for cholera).

Synthetic Subunit Vaccines.—Much current work is directed toward the development of vaccines consisting of uniquely specific antigenic molecules of completely known structure. It is hoped that such materials, of uniform high quality, can be synthesized in production volumes, providing inexpensive, safer, and more effective products.

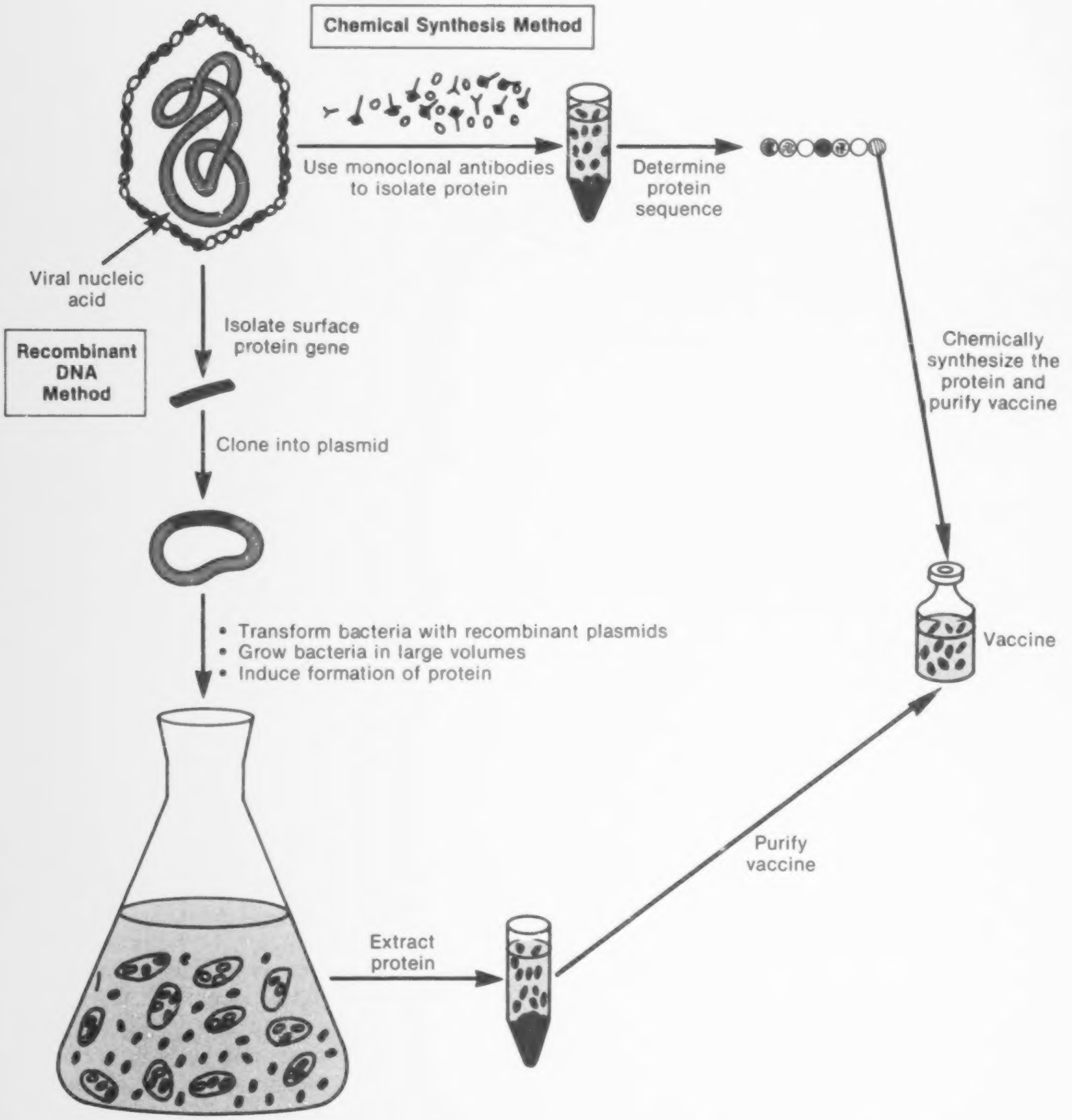
1. **Recombinant DNA products:** Three strategies using recombinant DNA technology are useful in synthesizing vaccines:

   a. Antigenic substances that stimulate protective immunity are identified or extracted from the pathogenic organism. Appropriate segments of pathogen DNA, coding for production of those antigens, are spliced into the DNA of another organism. In some cases, the host organism is a yeast or bacterium which thereafter synthesizes the desired molecule. The protective antigen is then separated from other materials in the culture and prepared as a vaccine (see fig. 7-1).
   b. The host organism (from a above), if nonpathogenic, can be used to infect the vaccinee, who then produces an immune response against the harmless carrier organism and the "piggy-backed" antigen. The nonpathogenic carrier organism can be either a related strain of the disease-producing DNA donor or an unrelated species.
   c. A pathogenic organism can be "attenuated" by deleting the specific gene causing disease, thereby rendering the organism nonpathogenic but still antigenic.

2. **Total synthetic antigens:** If a specific, defined protein or polypeptide (amino acid chain) is determined to induce protective immunity, scientists may be able to produce this antigen in pure form by a total chemical synthesis (see fig. 7-1) and use it to induce an immune response (314).

3. **Anti-idiotype vaccines:** In order to make anti-idiotype vaccines, scientists produce a passively protective MAb against the disease organism, then produce a second MAb against the first MAb. The second MAb (in effect, an "anti-antibody") may then be used as an "antigen" to stimulate production of the original protective antibody in a host. This application of MAbs would be most significant where an immunogenic protective antigen is in short supply and not amenable to synthesis by recombinant DNA or chemical techniques.

Figure 7-1.—Methods Used To Prepare Subunit Vaccines Against Viral Diseases: The Recombinant DNA Method and Chemical Synthesis Method



Chemical Synthesis Method
Use monoclonal antibodies to isolate protein
Determine protein sequence
Viral nucleic acid
Recombinant DNA Method
Isolate surface protein gene
Clone into plasmid
Chemically synthesize the protein and purify vaccine
• Transform bacteria with recombinant plasmids
• Grow bacteria in large volumes
• Induce formation of protein
Vaccine
Purify vaccine
Extract protein

In the **chemical synthesis method**, proteins that comprise the viral surface are isolated, often with the use of monoclonal antibodies (MAbs). The protein sequence is then determined. Based on the sequencing information, large amounts of the protein or portions of the protein are made chemically for use as the vaccine.

In the **recombinant DNA method**, the gene that encodes the viral surface protein is isolated and cloned into an appropriate vector (e.g., plasmid), transformed into a host (e.g., a bacterium or yeast), and the host is grown in large quantities. Formation of the protein by the recombinant DNA and isolation of the protein results in the subunit vaccine.

SOURCE: Adapted from Office of Technology Assessment, *Commercial Biotechnology: An International Analysis*, 1984.

Culture of Disease-Producing Organisms

Major steps toward producing vaccines against tropical diseases have been made possible by recent advances in the culture of disease-producing organisms. A method for in vitro cultivation of *P. falciparum* (343), for example, removed a major roadblock to further research in malaria, namely, the requirement for maintaining this organism in animal hosts. Other advances include success in cultivating in vitro the complete life cycle of *Trypanosoma brucei*, which causes African sleeping sickness (156), and the discovery that armadillos are an excellent culture medium for *Mycobacterium leprae*, which causes leprosy. These technical advances have increased the availability of experimental material for research use, but further advances are still needed.

VACCINATION AND VACCINE RESEARCH: CURRENT STATUS FOR SELECTED TROPICAL DISEASES

Malaria

The development of a vaccine against malaria is one of the great challenges that is now being addressed. Steady progress over the past decade has encouraged the scientific community, and hopes for an effective vaccine within the next decade have been voiced. A detailed account of malaria vaccine development to the present appears in *Case Study B: The Development of a Malaria Vaccine*.

Human malaria is caused by four species of protozoan parasites of the genus *Plasmodium*: *P. falciparum, P. vivax, P. ovale, and P. malariae*. Other species of *Plasmodium* are infective to a wide variety of vertebrates other than humans (e.g., *P. berghei, P. yoeli, and P. chabaudi* to rats and mice, and *P. knowlesi* to rhesus monkeys).

Malaria parasites (*Plasmodium* spp.) have a complex life cycle (see ch. 4) with several distinct life-stages that could provide points of intervention for vaccines. One of the primary targets of current malaria vaccine research efforts is the sporozoite life-stage. Plasmodial sporozoites are introduced into a vertebrate host's bloodstream during the bite of an infected *Anopheles* mosquito. Once in the host's bloodstream, sporozoites move within a short time to infect the liver cells, initiating malaria infection. For a sporozoite vaccine to be effective in preventing malaria in humans, every sporozoite would have to be killed.

Another target of malaria vaccine research are the erythrocytic or blood stages—either schizonts within red blood cells or free merozoites—of the malaria parasite. Merozoites, the stage most directly responsible for the symptoms of human malaria, develop from sporozoites in the infected host's liver cells (exoerythrocytic cycle), burst out of the liver cells, enter the host's circulatory system, and invade the person's red blood cells (erythrocytes). There they undergo asexual reproduction, forming schizonts, which release more merozoites to infect more red blood cells (erythrocytic cycle). In chronic infections, this cycle may be repeated many times. Merozoites are the main target of blood stage vaccine research.

A third target of malaria vaccine research is the gamete, the sexual stage of the parasite. Gametes mature in the mosquito from gametocytes that are picked up when the mosquito bites an infected person. If the person had been vaccinated against the gamete stage, the mosquito would also pick up antibodies circulating in the host's blood formed against the gamete. In the mosquito, the human antibodies would prevent the gametes from completing their life cycle, and thus prevent the mosquito from spreading malaria. Since the gamete vaccine would not protect the vaccinee from acquiring malaria, this type of vaccine is called an "altruistic" vaccine.

Two other potential vaccination targets are the infected liver cells and the infected red blood cells, whose surface membranes may be altered by the presence of parasites in the cell.

Most evidence indicates immunity is stage-specific in malaria. A vaccine against merozoites released from red blood cells, therefore, will not control exoerythrocytic merozoites released from liver cells (291). Successful immunization in human populations will probably require a combined vaccine against several stage-specific determinants, although a monovaccine against the sporozoite stage may have value to short-term visitors to areas where malaria is endemic.

Early Attempts at Immunization

Experimental malaria immunization was achieved using sporozoite, merozoite, and gametocyte vaccines during the late 1960s to mid-1970s.

Sporozoite Stage.—Immunization with radiation-attenuated sporozoites was shown to give excellent protection in rodents, birds, and human volunteers (19,61,62,261,262). One problem with this sporozoite vaccine is the difficulty of obtaining enough antigenic material in a sufficiently purified form to immunize even selected populations.

Erythrocyte Stage.—Vaccination with merozoites was achieved in rodents, birds, and nonhuman primate models using crude whole parasite antigens or partially purified antigens, but the use of adjuvants (nonspecific stimulators that enhance the immune response) was required (65,67, 68,241,242). (Because of side effects, especially tissue death at the injection site, at least some adjuvants cannot be used in humans.) Vaccination of rhesus monkeys with merozoite preparations of *P. knowlesi* in Freund's complete adjuvant conferred complete protection against an otherwise fatal infection. Immunity to blood stage parasites was also induced in *Aotus* monkeys with *P. falciparum* and *P. chabaudi* in mice. Various antigen preparations were used in these experiments: whole blood-stage *P. falciparum* parasites with adjuvant; merozoites obtained by "natural release" methods; and glutaraldehyde-fixed and freeze-thawed merozoites. Purified antigens of *P. yoeli* merozoites induced protection in mice. Rhesus monkeys were also vaccinated with an immunogenic protein isolated from the membrane of *P. knowlesi*-infected red blood cells.

Gamete Stage.—Gamete vaccination was demonstrated in chickens and rhesus monkeys. The mechanism of action is transfer (when the mosquito feeds) of antigamete antibodies produced in the animal host which act on the gametes in the mosquito gut (46,142).

Cultivation of *Plasmodium*

Although the possibility of protective immunization against malaria had been established by the mid-1970s, major progress towards producing vaccines required advances in the culture of malaria parasites.

In 1976, Trager and Jensen (343,344) described a method for in vitro cultivation of *P. falciparum* blood stages that removed a major roadblock to further research in malaria. Previously, malaria research had depended on maintenance of the parasite in animal hosts. This situation was unsatisfactory for several reasons: 1) it is expensive to keep animals; 2) large-scale production and isolation of the parasite is usually difficult; 3) biological phenomena of the host affect the malaria parasite and contaminate and increase the variability of any preparations.

In vitro cultivation of *P. falciparum* has become standard laboratory procedure to provide researchers with sufficient parasite material to perform other laboratory research on malaria. Because research on *P. falciparum* is most urgent (because of fatal consequences in *P. falciparum* infection), it was fortuitous that cultivation of *P. falciparum* erythrocytic stages proved easier than other malaria species. Success in cultivating merozoites of the other human malaria species is still elusive.

The next stage of the malaria parasite to be cultivated in vitro was the gametocyte, which when fed to mosquitoes through artificial membranes, led to the development of infective sporozoites (42). This was important for subsequent work on the sporozoite antigen because it ensured adequate amounts of material for study.

Liver stages of the parasite are now also being cultivated in vitro. The complete exoerythrocytic cycle of *P. berghei* (a mouse malaria) has been cultivated in the laboratory (158) (in a cell line of human embryonic lung cells). Attempts to cultivate the exoerythrocytic stages of monkey and human malaria are in progress. The tissue culture system *P. berghei* provides an in vitro assay to test for protective antibodies. It also provides a model for study of possible chemotherapeutic compounds.

Recent Research

Sporozoite Vaccine Research.—Using the *P. berghei* mouse malaria model and the in vitro tissue culture system, scientists have made important progress in the study of protective sporozoite antigens (63,422). The surface of the mature sporozoite is covered by a major species- and stage-specific membrane protein, the "circumsporozoite protein," which for *P. berghei* is named Pb44 for its molecular weight of 44,000. Synthesis of the circumsporozoite protein is a major metabolic activity of the mature sporozoite (immature forms of the sporozoite isolated from the mosquito midgut do not have this circumsporozoite protein, nor do later erythrocytic stages).

A very small quantity of purified MAb to Pb44 protects mice against sporozoite challenge (passive immunity). If sporozoites are incubated in vitro with the complete MAb or the antibody-specific portion of the MAb known as the "Fab" fragment, they lose their ability to infect liver cells (279). The antibody prevents attachment to, and penetration of, the mammalian target cells by *P. berghei* sporozoites in vitro (422). Thus, immunity to sporozoites in mice depends on binding of antibody to the malaria surface protein, preventing interaction with target cells.

Sporozoites infect liver cells by first attaching to a receptor on the cell membrane and then penetrating by means of a process involving movement between parasite and host cell (3). Attachment requires a protein receptor, and penetration requires movement of host cell components. Irradiated sporozoites attach, enter, and transform to the next liver stage (trophozoites), but do not develop to the mature liver (schizont) stage.

MAbs, which permit the recognition and isolation of individual antigens in pure form, have identified protective antigens from several species of *Plasmodium* (*P. knowlesi*, *P. chabaudi*, *P. falciparum*, *P. vivax*), including polypeptides analogous to Pb44 (64,89,431).

Building from the availability of sufficient antigen and a suitable model, scientists have now applied recombinant DNA technology to the production of pure protective sporozoite antigens; biosynthesis of the antigen by expression in *Escherichia coli* of the gene coding for protective sporozoite surface antigen has been achieved (109,432). The immunogenic region of the antigen has been identified and chemically synthesized (134,434). This work has been reproduced with the *P. falciparum* gene. Recently, the antigen produced by this process has been shown to be protective against *P. falciparum* challenge.

Erythrocyte Vaccine Research.—Erythrocyte antigens are being studied in rodent and primate malaria models and in *P. falciparum*. This research has been successful in producing scientific knowledge, but the implications for a blood stage vaccine are not yet clear.

Species- and stage-specific antigens have been identified on the surface of malaria-infected red blood cells (*P. chabaudi*, *P. knowlesi*, *P. falciparum*), and MAbs to at least one antigen showed protective activity (*P. chabaudi*). Membrane antigens of *P. knowlesi* and *P. falciparum* have been compared; clinical immunity to *P. knowlesi* correlated with the presence of antibody to a particular molecule (MW 74,000), which was then isolated and used to immunize monkeys. Antibody to this protein inhibited in vitro multiplication. Thus, there is encouraging evidence that a parasite-derived antigen(s) is at the erythrocyte surface, and it stimulates protective immunity. Recombinant DNA technology is being applied in several laboratories to produce protective erythrocyte stage antigens (16).

However, antigenic variation has been shown in *P. falciparum* in *Aotus* monkeys (159,160). Strain-specific determinants on the surface of red blood cells infected with late-stage parasites were recognized by immune serum. These determinants varied according to host factors. Variant antigens can be identified by surface immunofluorescence, and protection is correlated with the presence of antibody to surface determinants. MAbs against geographic isolates of *P. falciparum* can distinguish between isolates, and some can block merozoite invasion of red blood cells.

These findings imply that functionally important antigens, which appear to protect by inducing antibodies, do exist in the erythrocytic stages, but differ between *P. falciparum* isolates, and that *P. falciparum* has the ability to vary surface antigens in response to the body's immune response. The possibility of developing a protective immunization against the merozoite stage appears to be rather poor, if the antigen that stimulates protective antibodies varies not only over place, but also over time. On the other hand, if there is one or more determinant common to all or most strains of the parasite, in spite of the variability, then a vaccine may be feasible.

One fact that may be exploited for a merozoite vaccine is that merozoites recognize receptor "glycoprotein" molecules on red blood cells. These glycoproteins play an indirect role in the invasion of red blood cells.

Other Immunologic Research.—Mechanisms of immunity are being investigated, and greater understanding of cell-mediated immunity (T-cells, macrophages, natural killer cells) has been achieved. Generalized macrophage activation, induced by substances unrelated to malaria, provides some protection to mice from *P. berghei* infection, perhaps by increasing phagocytosis of parasitized red blood cells (334).

Researchers at the National Institutes of Health (NIH) have demonstrated that *P. falciparum*-infected red blood cells attach to the cells that line blood vessels (endothelial cells) and to cells of a particular melanoma (skin cancer) cell line. This is confirmation of the longstanding hypothesis that *P. falciparum* somehow differs from the other species by being "sticky" and thus adheres to and clogs the blood vessels. This in vitro model allows further investigation of this phenomenon, especially in relation to the pathogenesis of cerebral malaria. The implication for vaccine research is that somehow the surface of malaria-infected red blood cells are different from normal red blood cells, a difference which perhaps can be exploited to selectively eliminate these cells.

Tropical splenomegaly syndrome (TSS), a complication of malaria infection, found especially in children in endemic areas, is being studied, and evidence obtained indicates that antibody responses in TSS are specific to malaria. Since TSS may be some type of immune disease brought on by the body's response to infection, it must be considered and evaluated in the development of a vaccine.

The immunosuppressive effect of extracts of malaria parasites is being investigated with the *P. berghei* model. Certain components of malaria parasite chemical extracts have been shown to suppress T-cell-dependent immune responses to other pathogens. This finding may help to explain why malaria infection lowers the body's immunity to other infections and reduces the immune response to immunization for other diseases (e.g., measles vaccine). Further research in this area is needed.

Schistosomiasis

Schistosomiasis affects about 200 million people in parts of Africa, the Caribbean and South America, and the Orient. The major schistosomes infecting humans (*Schistosoma mansoni*, *S. japonicum*, and *S. haematobium*) have life cycles requiring certain freshwater snails as intermediate hosts.

Immunologic Research

Studies of schistosome infection using immunologic techniques have provided much new information of relevance to immunology itself, such as the first knowledge about the biological function of eosinophils (a type of white blood cell) and one of the immunoglobulins. In fact, it is only a slight overstatement to say that schistosomiasis has done more for immunology than vice versa.

The eosinophil is one of several types of white blood cells involved in cell-mediated immunity. The schistosome model is the most extensively studied laboratory system with respect to eosinophil involvement in immunity. There is mounting evidence that acquired resistance in schistosomiasis involves an antibody-dependent (humoral) mechanism that somehow activates the eosinophil to kill the foreign organism (19). The schistosomulae (the earliest life-stage of the schistosome in the body of the human host) appears to be open to attack by two mechanisms: 1) schistosomulae are coated with IgG antibody and then are attacked by eosinophils (39); and 2) IgE antibody-coated schistosomulae are attacked by activated macrophages, specialized cells of the immune system (44).

In addition to functioning at sites of skin penetration by schistosome larvae, eosinophils predominate in granulomatous lesions which develop around schistosome eggs entrapped in host tissues. The egg granuloma has been shown to be a principal factor in the pathogenesis of liver and spleen involvement in schistosomiasis, and histologic studies have indicated that the eosinophils destroy schistosome eggs. If eosinophils are eliminated by administration of anti-eosinophil serum, there is marked reduction in size of egg granulomas and concomitant decrease in pathology. This is an example of pathological damage to the human host arising from the host's own immune function.

Many laboratories have produced antischistosomal MAbs for identification of relevant antigens or for species identification (e.g., 83,84,99, 234,236,332,336). Dissous and colleagues (96) have reported protection against *S. mansoni* challenge in rats achieved by passive transfer of a rat antischistosomal MAb. This MAb recognized an antigen on the surface of immature worms and a different antigen on adult worms, both antigens sharing a common epitope. Other investigators have also reported passive protection against *S. mansoni* challenge, using monoclonals in mice (320,436).

Current Status

A live irradiated larval vaccine for schistosomiasis has been administered to cattle in the Sudan with encouraging results (338), but it is not acceptable for use in humans (because the larvae must infect the host, even though they do not mature to egg-producing adults).

Prospects for a subunit vaccine for schistosomiasis are not clear. Immunologic research has demonstrated the complexity of the immune response to the parasite and the parasite's protective responses. Developing schistosomes acquire host antigen on their surface, which masks them from the host immune response (130). The adult worm is largely protected from immune attack by acquisition of host antigen, shedding of antigen, and/or antigenic variation. The adult schistosome worm can survive for many years, apparently immune to host attack, yet stimulating immunity against further infection by larval schistosomes, thus becoming a chronic infection. Most evidence points to the early schistosomulae (the first immature infective stage) as the most likely point of attack for a vaccine.

Trypanosomiasis

Trypanosomiasis is a group of clinically differing diseases of the blood and tissues caused by several species of the genus *Trypanosoma*. The important human diseases are: African sleeping sickness (African trypanosomiasis), an acute form caused by *T. brucei rhodesiense*, and a chronic form caused by *T. b. gambiense*; and Chagas' disease (American trypanosomiasis) caused by *T. cruzi*.

African Sleeping Sickness (African Trypanosomiasis)

Current Status.—Currently, there are no vaccines against African sleeping sickness. One of the larger research efforts in vaccination for African trypanosomiasis is being carried out at the International Laboratory for Research on Animal Diseases (ILRAD) in Nairobi, Kenya, in an effort to prevent a similar disease in cattle (nagana) that has a devastating economic impact. The Special Program for Research and Training in Tropical Diseases (TDR) has a very modest effort that defers to ILRAD's effort.

Recent Progress.—In the past few years, scientists have made significant advances in demonstrating genetic mechanisms of antigen switching in African trypanosomes, with corresponding regulation of the expression of variant surface glycoprotein (VSG) genes. This work is important to vaccine development because surface proteins are the most likely antigens to stimulate the immune system.

Research on VSGs is now being pursued intensively, and it appears that at least two distinct mechanisms operate to cause shifts in VSGs: 1) changes in the gene itself, including duplication of a preexisting invariable basic copy of the VSG gene, followed by transposition of the newly duplicated "expression-linked copy" to an area of the genome in which it can be expressed; and 2) changes in gene activation related to a small segment spliced on to one end of the corresponding messenger RNA. Several types of genomic rearrangements have been described (22). Complementary DNA (cDNA) has been prepared for a variety of VSGs. Scientists have found that the VSGs are encoded by a fairly large number, perhaps up to 1,000, separate genes. DNA sequencing has been done on several VSG genes, which show considerable similarities over a large segment (290). The complete nucleotide sequence of cDNA coding for a VSG of *T. brucei* has been worked out by Boothroyd and colleagues (25), and the manner in which the VSG is made from more complex precursors is becoming known.

Current work on African trypanosomes by American investigators is centered largely on the molecular biology of antigen switching of VSGs. Roughly half a dozen laboratories are working intensively on mechanisms of transcription and expression, the nature of requisite messenger RNA splicing, evolution of the VSG repertoire, and similar problems. Although some workers are attempting to characterize the complete antigenic repertoire of African trypanosomes and are searching for nonvariant antigens across strains, there is little hope of developing a vaccine in the near term. The hope is that this research will pay off in the short term by identifying discriminatory proteins for use in immunodiagnosis and metabolic targets for chemotherapy.

Chagas' Disease (American Trypanosomiasis)

Current Status.—The current status of vaccination for Chagas' disease is generally considered unpromising. Experimental vaccines have been made by conventional methods (339) but have not been widely adopted. *T. cruzi* appears to be a good candidate for application of MAbs to develop vaccines. Immunization against *T. cruzi* is complicated, however, by the possibility that the severe immunopathologic events of Chagas' disease may be exacerbated by a vaccine.

Wood and colleagues (410) have reported production of a MAb against rat nerve tissue that cross-reacts with *T. cruzi*. Thus, a trypanosome antigen, which is identical to, or close to, a protein that is in host tissue, may be important in the pathology of this infection. It may be that the host immune system is stimulated to produce antibody against the parasite, that coincidentally cross-reacts with host tissue in a type of autoimmune response. The implication is that a vaccine that stimulates a protective antibody may stimulate immunity and pathology at the same time.

Recent Progress.—MAbs to culture and intracellular stages of *T. cruzi* have been made by several workers. Araujo and colleagues (10) reported production of 17 different secreting hybridomas, of which 5 recognized the antigens of intracellular stages only, 2 recognized culture forms only, and 10 reacted with both. Immunofluorescence procedures pinpointed the specific antigenic sites on the *T. cruzi* organisms. Snary and colleagues reported production of MAbs recognizing different stages, but not different species of trypanosomes (323), demonstrating the presence of stage-specific determinants.

In a remarkably original experiment, Crane and Dvorak (80) reported the fusion of *T. cruzi* organisms directly with vertebrate cells using a procedure analogous to hybridoma cell fusion. Three hybrid clones continued to express *T. cruzi* antigens for at least 14 weeks of serial cultivation, suggesting the continued presence of functional parasite DNA. A great deal of research activity with MAbs is under way. The research is aimed at identifying protective antigens which may be suitable for use in a vaccine, but progress is still elusive.

An animal model for Chagas' disease that mimics the human disease is being pursued by several laboratories. The objective is to have an experimental system for evaluating immunologic responses to *T. cruzi* and the protective effect of any candidate vaccines.

Research Needs.—A vaccine against Chagas' disease is badly needed, but researchers are not highly optimistic that one can be developed (16). In lieu of a vaccine, one investigator demonstrated some protection in mice by passive immunization with a MAb, suggesting that a different immunologic approach to parasite reduction may be feasible (16). Improvements in drug therapy for

Chagas' disease, rather than vaccination, may be a more urgent and feasible priority.

Leishmaniasis

Leishmaniasis is caused by several species of the protozoan genus *Leishmania*. Different species cause different clinical diseases:

1. cutaneous leishmaniasis;
2. mucocutaneous leishmaniasis, which begins as a skin lesion that progresses to destruction of tissue and cartilage of the face, nose, and throat; and
3. a visceral form of leishmaniasis known as "kala azar."

Current Status

Leishmaniasis vaccination using living cultured organisms has been attempted since the early years of the 20th century, and field trials with a frozen whole organism vaccine have recently been undertaken in Israel (138). Experimental studies in Australia using inactivated whole *Leishmania* (frozen and thawed infected culture cells) to infect genetically resistant and susceptible strains of mice are under way to devise standardized vaccination protocols. It has been demonstrated that avirulent parasite strains can induce protection in mice against related virulent strains (353). The theoretical possibilities of immunization are being developed and evaluated.

Research Needs

In view of the large number of strains and types of *Leishmania* and scientists' limited ability to identify organisms, there is doubt about whether a leishmaniasis vaccine will ever be practical. Each isolate is different. However, there is encouraging evidence from animal experiments and practical evidence of the potential for inducing active immunity. Further research on the current leads is needed.

Filariasis

Filariasis is the collective term for several distinct parasitic infections by insect-transmitted, tissue-dwelling nematodes (roundworms). The most important worldwide are *Wuchereria bancrofti* and *Brugia malayi*, the agents of filarial elephantiasis; and *Onchocerca volvulus*, the agent of onchocerciasis (river blindness) in west Africa, also found in Central and South America.

Current Research

Until recently, filarial organisms were difficult to maintain for laboratory study, with complicated life cycles requiring insect vectors at one point. The lack of suitable animal models for many filarial diseases also hindered research. Investigators have achieved successful in vitro cultivation of *B. malayi* and a related species *B. pahangi* from the larval stage to young adult stage. They have also achieved some success with leaf monkeys (*Presbytis melalophos* and *P. cristata*) as experimental hosts (16).

Evidence of humoral immunity has been demonstrated by passive transfer of serum in mice and cattle previously inoculated with crude parasite preparations or live microfilariae.

Antifilarial MAbs are being produced for diagnostic purposes, but they can also be used for identification and purification of protective antigen. Attempts to identify specific antigens of possible significance to protection against *Wuchereria*, *Brugia*, and *Onchocerca* are under way. At least one U.S. Government laboratory, one industry laboratory, and one university laboratory are conducting or planning recombinant DNA work for expression of such filarial antigens (16).

Leprosy (Hansen's Disease)

Leprosy is the only bacterial disease among the six tropical diseases targeted by TDR. It is mainly a disease of skin and peripheral nerves, but is characterized by a wide array of clinical presentations.

The severe form of leprosy, lepromatous leprosy, occurs in patients with defective cell-mediated immunity. These patients develop massive, contagious infections with extensive nerve damage. The nerve damage in severe cases seems to result from an overactive cell-mediated immune response. Most patients, however, develop a high level of cell-mediated immunity which kills and clears bacilli from the tissues. These patients, with tuberculoid leprosy, have a good prognosis.

The role of humoral immunity in leprosy still is not clear, but may be detrimental to the host. In the process of isolating *Mycobacterium leprae* bacilli from armadillo tissues, investigators have obtained significant quantities of a phenolic glycolipid that is the only unique antigen present in *M. leprae* and not other mycobacteria. In vitro studies now indicate that immune reactions to this antigen may actually increase disease, without producing a protective effect (222). Characterization of the immune defect in lepromatous leprosy patients is being investigated using techniques of biotechnology and also by analysis of the patients' genetic makeup. Understanding the immune response to infection with *M. leprae* will help in developing immunization strategies.

Current Research

The tuberculosis vaccine BCG (an attenuated strain of a bovine mycobacterium) has been tested for an immunoprophylactic effect in large-scale prospective trials in Uganda, Burma, Papua New Guinea, and India. Long-term followup in these trials indicated a variable and not very effective protection against leprosy.

An interesting development in leprosy vaccination is the work of Convit and collaborators during the past decade in Venezuela (72). These researchers conducted clinical trials in a variety of individuals, including some with early disease, using a vaccine made of heat-killed *M. leprae* (grown in armadillos) combined with BCG vaccine. They found clinical, histopathologic, and immunologic changes in many patients with disease of moderate severity. The investigators' claim of conversion to effective cell-mediated immunity in lepromatous leprosy patients and more rapid clearance of bacilli in patients treated needs confirmation. Vaccine field trials using killed *M. leprae* only, BCG vaccine only, and *M. leprae* with BCG vaccine are planned. Such trials will require considerable amounts of *M. leprae*. Since leprosy is a disease of low incidence and long incubation period, the evaluation of efficacy of a vaccine will require 10 years or more of observation in large populations.

Carefully controlled human trials of leprosy vaccine from TDR are now under way in Norway in individuals never exposed to leprosy. It remains to be seen whether this vaccination will simply mimic natural exposure to leprosy (i.e., stimulating cell-mediated immunity in most people, without helping the smaller proportion of people who cannot mount such a response), or if it will support the earlier results of Convit and colleagues (72) of having some effect even in the lepromatous leprosy patient.

Extensive and careful planning of the production, purification, and testing of the leprosy vaccine, plus selection and epidemiological characterization of test populations has been under way in the TDR project for several years (349,350,353).

At least two groups are planning recombinant DNA work to try to make a leprosy vaccine, in order to eliminate the need for recovering *M. leprae* from armadillos (72). Some workers feel that subunit vaccines are not likely to confer significant protection, since they induce a humoral response but not cell-mediated immunity. One group of workers has isolated and characterized DNA from *M. leprae* and investigated its relationship to other mycobacteria (170). Further work may identify important DNA segments that could be cloned and used to produce quantities of a specific antigen.

Research Needs

Obtaining antigens from *M. leprae* grown in armadillos is a slow process and not suited to producing large quantities. In vitro culture methods are needed to facilitate leprosy research.

A concentrated effort is needed to use recombinant DNA methods and T-cell clones to sort out *M. leprae*-specific epitopes pertinent to protection. Better knowledge of cell-mediated immunity and the cellular aspects of host reactions is needed.

Further evaluation of BCG vaccine and *M. leprae* antigens through immunization trials is continuing. The fact that the tuberculosis vaccine (BCG vaccine) may prove to be useful against leprosy points out the importance of further study of the relationship of *M. leprae* to other mycobacteria, especially *M. tuberculosis*.

Tuberculosis

Tuberculosis remains a major threat to health in many parts of the world, causing several million deaths annually. Even in the United States, some 30,000 new cases of pulmonary tuberculosis were reported in 1980 (70). There is a tuberculosis vaccine, BCG vaccine, but its efficacy is uncertain and controversial. (For a review of immunology and microbiology of tuberculosis, see Collins (70) and Wayne (398).)

Current Status

BCG vaccine is an attenuated strain of a species of bovine mycobacterium. Since the early 1950s, BCG vaccination has been used extensively in tuberculosis control programs around the world. Even in the 1950s, however, it was known that BCG vaccine did not offer complete protection against tuberculosis. Results of controlled field trials were contradictory, with protection varying from 0 to 80 percent.

All the BCG vaccines used throughout the world today are derived from the original strain developed at the Pasteur Institute more than 50 years ago. There is no reliable method for standardization of BCG vaccines. Most people agree that the probable variation of different vaccine preparations, compounded by differences in immunologic response of populations, is the main reason for the enormous discrepancies in results between otherwise well-conducted BCG trials.

Because of the lack of definitive evidence of BCG's efficacy, a controlled double-blind field trial in southern India was started in 1968 (414). Two highly ranked vaccines were used. After 7½ years of careful followup, there was no evidence of a protective effect in BCG-vaccinated groups. The Indian field trial was meticulously reviewed (415,417) and found methodologically sound. Quite possibly, the explanation for the results in this trial is that the population had already developed some resistance to tuberculosis through exposure to a widespread south Indian nonhuman mycobacterium; and BCG vaccine could not add to that resistance. The results from this trial may not be applicable to other parts of the world, depending on local conditions. BCG vaccine is still considered useful in tuberculosis control programs.

Immunologic research on tuberculosis has been given relatively little attention over the years, and the field trial results in southern India clearly revealed a large gap in knowledge about the immunology of tuberculosis (417). Application of recombinant DNA methods to *M. tuberculosis* is in its infancy, but several investigators are planning projects. MAb work is under way in a handful of laboratories, including some at NIH and several academic institutions. MAbs are being reacted with *M. tuberculosis* to obtain purified antigen probes. Attempts are being made to isolate specific antigens for diagnostic skin tests (see ch. 8) in an attempt to make such tests less cross-reactive to infections with other types of mycobacteria, but such antigens may also have value for immunization.

Research Needs

A great deal of fundamental knowledge about tuberculosis immunology is needed to resolve the pending questions about immunization in general, the efficacy of BCG vaccine, and the susceptibility of various related mycobacteria to vaccination and their importance in the disease process.

Better understanding of tuberculosis immunology and vaccination and clarification of the effectiveness of BCG vaccine are still needed.

Diarrheal and Enteric Diseases

Diarrheal diseases are caused by a variety of viruses, bacteria, protozoa, and worms. Development of water and sanitation facilities where they do not now exist is a long-term solution to control these diseases. Nevertheless, some of the agents of diarrheal disease may well be susceptible to immunologic attack, thereby permitting prevention of disease with vaccines.

A few injectable vaccines inducing serum antibodies have been available for years (for cholera, typhoid, paratyphoid, *Shigella*), but these have limited effectiveness. Oral vaccines, which in most cases appear to induce a more appropriate immune reaction in the gut, are currently the focus of much research.

Although some research will have cross-over potential for several agents, it is generally expected that each pathogen will need a tailored approach. Immunologic research has made it clear that the vaious etiologic agents evoke and are fought by a variety of immune responses. A further challenge of diarrheal disease research is the continuing discovery of new important etiologic agents.

Viral Infections

Rotaviruses cause about one-third of all diarrheal disease in the world and up to 50 percent of the hospitalized cases of diarrheal illness in children under 2 years (21,167,325,331). Animal rotaviruses are also important because of their economic significance in farm animals, and some veterinary vaccines have been developed and are in use.

Characterization of the rotavirus genome is being actively pursued with the aid of recombinant DNA techniques. Rotavirus RNA has been synthesized in vitro, reverse transcribed, and inserted into *Escherichia coli*; the genes from which most of the clones derive have been identified (117); bacterial clones containing copies of each rotaviral gene have been identified; some of the genes have been analyzed to determine the amino acid sequence of the proteins for which they code. This work is a promising means of identifying critical genes coding for the pathogenic characteristics or antigenic determinants of the organism. If successful, production of a strain of rotavirus with deletions of the pathogenic genes (similar to the cholera research described below) or transfer of the DNA segments that specify the antigenic determinant(s) and production of the antigen may be possible, forming the basis of a vaccine.

A major obstacle to vaccine development has been the lack, until recently, of a cell culture system for human rotaviruses (192,429). It may now be possible to develop attenuated vaccines through the various conventional methods (cell culture passage, cold adaptation, chemical mutagenesis, and reassortment) (371,385). In fact, volunteer studies in adults are being conducted to provide a test system for such candidate vaccines (181,182).

Animal rotaviruses have been successfully grown in culture, and attenuated strains are available as veterinary vaccines. Studies in calves, piglets, and lambs have demonstrated the importance of intestinal rotaviral antibody in preventing or attenuating illness. Because human and calf rotavirus strains share a common group antigen (179,711), calves inoculated in utero with bovine rotavirus were protected against challenge with human rotavirus. Furthermore, piglets infected with bovine rotavirus and later challenged with human rotavirus showed cross-protection (204,205).

On the basis of this evidence, an oral, live, attenuated bovine rotavirus vaccine was recently tested and found protective in a test population of human adults and children (385). The vaccine has now been tested and found to be safe and effective in protecting infants from natural rotavirus infection (386). This is a very promising development that is being tested further.

Bacterial Infections

Most of the vaccine research on the bacterial enteric pathogens is being done on enterotoxigenic *E. coli*, *Salmonella typhi* (the cause of typhoid fever), *Vibrio cholerae* (the cause of cholera), and *Shigella* (the cause of bacillary dysentery). Most research groups are studying several of these agents. Fewer laboratories are studying *Campylobacter*, *Yersinia*, other species of *Salmonella* and *Vibrio*, and other pathogenic intestinal bacteria. Approaches to vaccine development are generally similar to those described below for *E. coli*. Progress against several of these agents is described below.

Coliform Infection.—Various strains of *E. coli* are now recognized as major causes of diarrheal disease in older children and adults living in developing countries. They also appear to be a chief cause of "traveler's diarrhea." The disease-causing characteristics of *E. coli* are under genetic control, and investigators in several laboratories are actively identifying and cloning the genes that code for attachment and colonization, virulence, toxin production, and antibiotic resistance. The goal of this work is the development of strains that stimulate specific immunity but are not themselves pathogenic. Successful development of vaccines against enterotoxigenic *E. coli* in animals has stimulated work towards a vaccine for humans (427).

Typhoid Fever.—Injectable, killed *S. typhi* vaccines against typhoid fever have proved to be protective for adults and older children living in endemic areas. These vaccines are unsatisfactory, however, because they frequently induce adverse reactions, they are not entirely protective, and they do not stimulate local intestinal immunity (419).

While oral, killed typhoid vaccines have provided only minimal protection in volunteer and field trials, several live attenuated *S. typhi* strains are under study and good progress has been made. A strain developed by the Swiss Serum and Vaccine Institute may well become available for widescale use very soon. This strain has been tested for stability, safety, and efficacy in volunteers (127), and in field trials in Egypt (391), all with positive results. The results of a trial in Chile, in collaboration with Walter Reed Army Institute of Research, unfortunately do not match the Egyptian results (24). The WHO Program for the Control of Diarrheal Diseases is planning for the use of this vaccine by collecting baseline information on the incidence of typhoid fever in different countries and reviewing other relevant data (423,427).

Cholera.—Cholera is relatively rare compared to the other diarrheal diseases, especially those caused by rotavirus and *E. coli*. However, the epidemic and pandemic potential of cholera is great enough to make cholera an important tropical disease. The severe diarrhea of cholera is caused by a toxin produced by *V. cholerae*, acting on the gut wall.

Although injectable, killed whole-cell and toxoid cholera vaccines are currently in use, these vaccines do not stimulate effective, long-lasting protection against cholera (47,86,200,201,260).

Scientists using traditional nonrecombinant mutagenesis have produced a number of attenuated mutant strains of *V. cholerae* that do not produce the cholera toxin (nontoxigenic strains). These attenuated, nontoxigenic strains have been used experimentally as oral vaccines with some encouraging results, but genetic instability or poor colonizing ability in the gut make them unsuitable.

Researchers have very recently succeeded in using recombinant DNA techniques to produce a live, attentuated *V. cholerae* strain by deleting gene coding for the cholera toxin (178,223). A recombinant strain such as this could provide a vaccine that colonizes the gut and stimulates immunity without producing the toxin and without the capability of mutating back to the pathogenic type. Very early clinical studies are under way to assess the safety and efficacy of this new vaccine. Unfortunately, the *V. cholerae* with the genes for cholera toxin deleted cause diarrhea in volunteers, presumably from a different toxin (230).

Shigellosis.—*Shigella dysenteriae* is associated with serious disease and fatality (bacillary dysentery), but *S. sonnei* and *S. flexneri*, which cause less severe disease, account for a major portion of all isolates in diarrhea patients. The available injectable, killed vaccines are not effective, and oral vaccines have not been developed until recently. *Shigella* will colonize primates only, making work with animal models difficult and expensive. One group of researchers has taken an attenuated, live typhoid vaccine bacterium developed recently in Switzerland and has spliced into it the genes coding for production of an important *S. sonnei* antigen, thereby inducing immunity to both typhoid fever and *S. sonnei* dysentery to vaccinees (126,123). Testing in humans is getting under way. The same group has isolated genes for attachment factors in *Shigella* and inserted these through plasmid vectors into noninvasive *E. coli*. These novel recombinations as well as a number of attenuated strains are under investigation as potential vaccines, but solid success is still elusive.

Acute Respiratory Infections (ARIs)

ARIs are a heterogeneous mixture of diseases, caused by a variety of viruses, bacteria, and other agents, some of which also cause infections outside the respiratory system. As detailed below, some vaccines for ARIs are available, and progress is being made in developing new vaccines.

Viral Infections

Many viruses cause acute respiratory illness in humans. The following groups have been identified as most important (see Anderson, et al. (5) for a recent extensive review):

Influenza.—Outbreaks of influenza may occur annually, major epidemics every 2 to 3 years, and pandemics at 10- to 15-year (or more) intervals. The last pandemic (as of July 1985) was in 1968. In pandemics, mortality rates are devastating, particularly in the elderly. Three main types of influenza viruses, with numerous subtypes and strains, cause infection in humans. Infection sometimes confers long-term strain-specific immunity or partial immunity. Because of antigenic drift and shift, influenza vaccines must be reformulated every year against the predominant virus. The annual production of influenza vaccine targeted for the strain predicted to be prevalent is now well implemented in the United States. However, the need for annual renewal makes mass influenza vaccination in developing countries impractical at this time.

Knowledge of the chemical and antigenic structure of influenza virus has increased greatly and, combined with the application of current molecular biology, is leading to better influenza vaccines. More rational approaches to vaccine development have emerged, e.g., the discovery of chemicals able to release surface glycoproteins from the viral particle without affecting their antigenicity, thus producing an efficacious and safer (fewer side effects) killed vaccine; the use of "high yield" influenza A viruses are being used to produce seed strains for better vaccine production (264).

Inactivated influenza vaccines are in use in developed countries. Killed vaccines administered by injection are made from virus particles disrupted by chemical treatment. This treatment lowers the antigenicity but also reduces the adverse side effects. Purified vaccines that contain only the immunogenic antigen, free of nonimmunogenic proteins, and are well tolerated by children can be produced (198,347). The vaccines are usually reserved for selected high-risk populations, such as the elderly, chronic disease patients, or economically important public service groups. Although these vaccines do not provide complete protection (225) and their effectiveness is dependent on annual renewal according to the prevalent strain (163), the Centers for Disease Control has conducted field studies that show inactivated influenza vaccine to be extremely useful in preventing or attenuating influenza infection (54, 374).

Live, attenuated influenza vaccines for intranasal administration are being developed. The attenuated vaccines can be rapidly and reliably produced by the transfer of genes from a cold-adapted attenuated donor virus to any new wild-type influenza isolate. Live vaccines, produced by reassortant RNA, eliminate the pathogenic genes but retain the immunogenic surface antigens. Recent research has indicated that these vaccines may be superior to the inactivated vaccines (60). In a study in adult volunteers, the live, attenuated vaccine completely protected against illness, while the licensed inactivated vaccine did not. Furthermore the nasal administration (by nose drop) is more convenient for patient and medical personnel. Even with the live, attenuated vaccine, however, there is a need for annual administration. Longer term protection will be possible only if there are common antigens among strains.

Much effort by 8 to 10 laboratories in the United States is focused on the molecular biology of influenza. This reflects, to a large extent, the importance of influenza as a public health problem in the United States. With immunity under active study (76) and the practical value of immunization under review (271,304), hundreds of publications in recent years have resulted. A large number of genes have been identified and sequenced. Synthetic peptides have been found to be antigenic and are undergoing evaluation, but preliminary studies have shown that such vaccines have greatly diminished capacity to induce neutralizing antibodies. In an effort to understand virulence factors, investigators have applied innovative methods, such as "tryptic peptide fingerprinting," "RNA-RNA hybridization," and "oligonucleotide mapping" (16).

One interesting development is the cloning of influenza genes in vaccinia virus (the smallpox vaccine) and the subsequent expression of both vaccinia and influenza antigen resulting in simultaneous immunization of hamsters against both agents (319). Because the vaccinia virus is well characterized, and several extra genes can be spliced into its genome, there is speculation that this experimental work could be developed into a method for delivering several vaccines in one organism. Many safety questions need to be answered before the vaccinia vector approach can be evaluated fully. The rate of adverse reactions from smallpox vaccine is higher than those for vaccines in current use, and may not be acceptable when smallpox is not the target.

Respiratory Syncytial Virus (RSV).—The several known strains of RSV are the most important causes of lower respiratory disease (pneumonia and bronchiolitis) in children under 2 years. The disease tends to occur in sharp outbreaks. Previous immunization efforts have not been successful, but new techniques show promise. Cloning of surface proteins or production of genetically engineered attenuated vaccines may meet with success.

Early work using inactivated RSV showed that vaccinated children developed antibodies, but were not protected against infection (180,186). Various immunologic phenomena have been hypothesized and studied without clear resolution.

Vaccination with attenuated RSV administered through the respiratory tract has not been successful. The degree of attenuation is variable, and the vaccines are not very stable (49,286). Injecting wild-type RSV grown in cell culture induced antibodies in young children without causing symptoms of disease (40). However, there is no evidence that RSV given by injection will reproduce, nor that the vaccine will not interfere with passively transferred maternal antibodies in infants. The lack of vaccine stability and the uncertainty of a viral replication after injection are important, because a virus that fails to reproduce may act as an inactivated antigen that can potentiate disease due to natural infection, and because the greatest need for effective RSV immunization is in the first months of life when maternal antibodies are still circulating in the infant (passive immunity). Another important finding that may hamper vaccine development is the report of antigenic variation in a new strain of RSV, which means that any single vaccine may be ineffective against some RSV strains (154).

Parainfluenza.—There are four main types (serotypes) and two subtypes of parainfluenza viruses. These viruses are the second most common agents of ARI (after RSV), producing croup, bronchitis, pneumonia, and bronchiolitis in infants and children.

Vaccine research thus far has shown that serum antibodies circulating in the blood against type 1 virus do not protect against infection, but antibodies in nasal secretions directly correlate with resistance to reinfection (317). Vaccines of formalin-inactivated virus administered by injection induce high titers of neutralizing antibody without preventing infection, though severe illness is prevented. Results with experimental live, attenuated vaccines administered intranasally have been encouraging. Whether killed or attenuated virus is used, the usefulness of a parainfluenza vaccine would have to be measured by the vaccine's protective effect in children, especially infants, who often become infected during the first months of life and suffer the most severe symptoms. Establishing the safety of a vaccine will be difficult (198,264).

Adenoviruses.—At least 41 different types of adenoviruses have been identified as causes of ARI (especially a problem in military recruits in the United States), as well as of epidemic keratoconjunctivitis (an eye infection) and a venereal disease.

Vaccines to prevent adenovirus infection have been developed for use in military recruits. A live vaccine cultured in human cell culture has replaced the earlier killed vaccine, which was highly protective but was inconsistent in different batches. The live vaccine, containing two different serotypes responsible for adenovirus infection in military recruits, is given orally (264).

Unfortunately, the experience with the recruits is not directly transferrable to the general population, for two fundamental reasons. First, the strains of adenovirus that cause most disease in recruits are different from those prevalent in the population at large. Second, while oral administration works for recruits, it is unsuitable for children, because recruits, in general, do not pass the vaccine virus on to each other, but children do, and a good deal of disease can be caused in that way.

Rhinoviruses.—Rhinoviruses, with more than 100 serotypes, are the most common human ARI agents and the single most important cause of the common cold.

Inactivated rhinovirus vaccines have shown some protection when administered intranasally, but not by injection. Live, attenuated vaccines may provide more protection, but the large number of serotypes, the fact that rhinovirus strains may not completely lose their infectivity (51), the indications of antigenic variation (120), and the possible development of genetic reassortant variants as a result of dual infection all hamper the future development of rhinovirus vaccines (264). In spite of the problems, some commercial firms are working on the rhinoviruses.

Coronaviruses, Coxsackieviruses, Echoviruses.—These viruses are important causes of common cold-like illness in children and adults. Coronavirus disease is self-limiting (about 7 days) and will not be a priority for vaccination. Coxsackieviruses induce a resistance after infection that is long lasting. With many different types prevalent in different areas and the predominant type in any area varying every few years as immunity in the population rises, there will be obstacles to vaccination. Echoviruses share epidemiologic features of coxsackieviruses. Immunization does not seem to be a practical method of preventing infection because of the large number of serotypes (129,264). There currently is little, if any, vaccine-related research on this group of viruses (16).

Measles.—Measles is an ARI for which an excellent vaccine exists. The vaccine is widely used in the United States, as part of a measles eradication program, and in other parts of the world, in particular, as part of WHO's Expanded Program on Immunization (see ch. 5). Measles vaccination has been controversial in developing countries because of the variable seroconversion rate (percentage of vaccinated individuals who later have measurable antibodies) and protection rates (percentage of vaccinated individuals who do not get the disease). A problem that has impeded success of vaccination programs is that infants must be vaccinated in the narrow interval between the waning of maternally derived antibody and the time of exposure to natural measles.

Recent clinical trials of live, aerosolized measles virus vaccines (298,299,300) have demonstrated that the intranasal route of administration may be particularly useful in developing countries. In contrast to vaccines injected through the skin, the aerosolized vaccine can induce seroconversion in the presence of maternal antibodies, in infants as young as 4 months. The aerosolized vaccine raises the respiratory tract antibody level, offering better protection at the portal of entry rather than in the serum. It also simplifies the logistics of vaccine delivery. A recent study indicates that not all measles vaccines stimulate an equally high level of protection in humans, even though they meet potency standards at manufacture (301). Better definition of potency standards is required. Further clinical trials are needed to assess the true efficacy of the aerosolized measles vaccine (4). In addition, the practicality of delivering aerosol vaccines in developing countries requires investigation. Getting the dosage right with aerosols, for instance, is much more difficult than it is with other methods of vaccination.

Bacterial and Mycoplasmal Infections

Streptococcus pneumoniae.—*S. pneumoniae* causes pneumococcal pneumonia, a disease that can be fatal when complicated with bacteremia (bacteria in the bloodstream). *S. pneumoniae* accounts for approximately 80 percent of all bacterial pneumonias; it is an important cause of hospital admissions in developing countries and an important cause of middle ear infections and bacterial meningitis (12,248,292).

Pneumococcal vaccine is available, and its protective effect is well characterized (356). Immunity is specific to each serotype (subgroup of a species characterized by common antigens), so vaccines must include the most common serotypes found in the area of use. The polyvalent formulation used for the vaccine contains capsular polysaccharides from a number of pneumococcal types. The vaccine is ineffective in children, especially under 2 years. It is usually targeted to older people whose risk of infection and case-fatality rates are greatest. Some new techniques of conjugation show promise in improving effectiveness in young children, but a major problem is the lack of information about which strains are prevalent in developing countries.

***Streptococcus pyogenes*.**—The approximately 70 serotypes of group A streptococci (*S. pyogenes*) cause a variety of diseases, including streptococcal sore throat, scarlet fever, and rheumatic heart disease. Attempts to produce a streptococcal vaccine have encountered two serious problems: cross-reaction of streptococcal antigens with heart tissue, and the large number of serotypes.

A synthetic peptide approach offers the possibility of eliminating cross-reacting antigens while preserving those components that confer immunity. A synthetic streptococcal vaccine has been successfully tested in animals, and development of a vaccine against rheumatic fever is possible within the next 5 years.

***Bordetella pertussis*.**—Pertussis (whooping cough) is endemic worldwide, and epidemics also occur. Children under 5 years of age have the highest incidence of morbidity, and about 70 percent of mortality is among children less than 1 year old (21). Immunization is available with a killed pertussis vaccine that is highly effective. This pertussis vaccine is usually given in a triple vaccine known as DPT (diphtheria, pertussis, tetanus). The use of DPT vaccine among children is promoted through WHO's Expanded Program on Immunization (see ch. 5). Intensive research to reduce or eliminate the adverse reactions seen with the current vaccine is under way.

***Hemophilus influenzae*.**—Immunization with the polysaccharide antigen of *H. influenzae* results in antibody response. Antibodies can also be induced by *E. coli*, which has a polysaccharide that cross reacts with *H. influenzae*. Attempts are being made to increase the immunogenicity of the antigen by coupling it to proteins (264). Prospects for licensing a new vaccine that is immunogenic in infants and children look very good.

***Mycoplasma pneumoniae*.**—*M. pneumoniae* is now recognized as a major cause of primary atypical pneumonia. Infection induces serum antibodies, but pneumonia can occur in individuals with positive antibody titers. While vaccination studies seem to demonstrate that serum antibodies correlate with protection, this and the role of respiratory tract antibodies still are not fully understood. Although antibodies appear in young children (2 to 5 years old), peak incidence of disease is in older children and young adults.

Field trials with inactivated *M. pneumoniae* vaccines have shown encouraging results, and attenuated vaccines have also been tested. However, some vaccinees have developed disease and have not produced detectable antibodies, and lung lesions may have resulted from an immune reaction. Further cautious work is needed to resolve these problems (50,264).

Arboviral and Related Viral Infections

Arboviruses include literally hundreds of distinct viruses that are widely distributed throughout the world. Arboviral infections such as yellow fever, dengue fever, and various types of encephalitis occur worldwide in endemic and epidemic forms.

Current Status

Vaccines are available for only a handful of arboviral infections. A live, attenuated vaccine for yellow fever (the 17D strain) was developed decades ago and is well established and successful in preventing yellow fever. It has proved to be extremely safe and effective. The immunity stimulated persists for more than 10 years. Yellow fever vaccine is grown in embryonated chicken eggs following classic procedures of viral vaccine production (373).

Formalin-inactivated vaccines are used against Japanese B encephalitis and Russian spring-summer encephalitis with good results. Experimental attenuated (Venezuelan equine encephalitis and western equine encephalitis) vaccines under research and development have been used in horses.

Research Needs

The most critical need is for prevention of dengue fever, not only because of dengue fever's debilitating clinical syndrome, but also because of dengue hemorrhagic fever (DHF), a serious complication of dengue infection that often leads to a shock syndrome and death (with symptoms similar to yellow fever).

Several laboratories, both military and civilian, are studying the biology of the four main serotypes of dengue virus. MAbs for early serotype-specific identification have been developed and are now generally available. These may also help to identify protective antigens. Attenuated vaccines are being developed using classical vaccine production techniques. Each of the four known serotype strains of dengue virus requires a separate vaccine. A serious question of vaccine efficacy relates to the origin of DHF. It is now believed that the sequential infection with two different dengue serotypes leads to DHF. It also appears that type 2 is the critical precipitator. Therefore, the hope is that vaccination against type 2 can prevent DHF; however, careful evaluation will be needed to ensure that vaccination will not lead to increased incidence of DHF as a side effect (143,144).

Very few vaccines exist against the arboviral agents, but previous successes argue for a continued research effort. Because there are literally hundreds of arboviruses that cause ill health, routine vaccination is still a long way off. Molecular characterization and increased taxonomic studies are needed to lead the way to "generic vaccines" (polyvalent) for large groups of related viruses, rather than serotype-specific vaccines. To develop vaccination strategies, there is also a need for better understanding of the epidemiology of arboviruses.

SUMMARY

Effective vaccines exist for some viral and bacterial diseases important in developing countries, but there are currently no antiparasite vaccines. The vaccines available in developing countries are largely those that are also used in the developed countries, mainly against childhood illnesses. Efforts to develop vaccines against rotavirus infection, which causes a large percentage of diarrheal illness, and malaria, one of the world's biggest health problems, are among the first efforts whose products will benefit mainly the developing countries, although there is some work in the direction of vaccine development for virtually every disease.

The successful application of immunization technologies, sadly, does not rest on exciting developments in research and development. The vaccines that are already available are far from universally applied because of financial and political constraints, and there is no reason to believe that new vaccines will fare any better unless they are supported by international and bilateral health programs.

8.

Diagnostic Technologies: Selected Tropical Diseases

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8.

Diagnostic Technologies: Selected Tropical Diseases

INTRODUCTION

Diagnosis has three important functions. One is to determine the nature of the individual's disease for the purpose of deciding on an appropriate course of treatment. A second function is to determine the prevalence of specific disease-producing organisms or agents in populations, to allow assessments of the impact of public health interventions. The third is to find out about the range of diseases affecting a population, the immune stages of populations, etc., for the purposes of research.

Diagnosis of tropical diseases is a challenge for the clinician or epidemiologist, because many of the diseases mimic a wide variety of infectious processes by presenting similar or ambiguous symptoms. In countries where these diseases are endemic, symptomatic ambiguity can also result from the common occurrence of multiple infections in a single individual. The proper identification of the species of the organism that is causing disease can be critical in clinical management, especially in serious illness.

Although each tropical disease can be diagnosed by a number of different methods, diagnostic techniques vary in their usefulness under different conditions. There are several parameters to consider in evaluating the usefulness of a diagnostic test: sensitivity, specificity, predictive value, cross-reactivity, and precision (388).

- *Sensitivity* is the ability of a test procedure to find a disease-producing agent or disease when it is present. A very sensitive test correctly identifies all infected individuals. Negative tests for individuals who have the disease are "false negatives."
- *Specificity* is the ability of a test procedure to correctly determine that a disease-producing agent or disease is not present. A very specific test will correctly identify all uninfected individuals. Positive tests for individuals who do not have the disease are "false positives."
- *Predictive value* is an interaction of sensitivity, specificity, and the prevalence of the infection in the population being tested. The sensitivity and specificity of a test will give different numbers of false negative and false positive results depending on the prevalence of the infection in the population tested. If a test is conducted in a group of patients strongly suspected of having the disease, very few false positives would be expected, simply because very few people tested will actually be negative. Thus, positive results will be readily accepted as true. Conversely, if the same test is conducted in a population in which very few people are thought to have the disease, the positives would have a higher probability of being false positives, and in fact, false positives may outnumber true positives. The negative test results will mostly be true negatives.
- *Cross-reactivity* is an aspect of immunodiagnostic tests related to, yet different from, specificity. A cross-reaction occurs when the test correctly identifies the appropriate chemical entity, but that chemical happens to exist in a different organism than the one tested for. A false positive results.
- *Precision* is reproducibility, the ability of a test procedure to give consistent results in repeated trials of the same sample.

Broadly generalizing, there are two categories of conventional diagnostic technologies:

1. Direct examination of blood, stool, urine, sputum, tissue biopsy, or cultured isolates

using simple equipment (e.g., a light microscope) and reagents.

2. Serologic examination to detect antibodies to the pathogen or to detect the pathogen or its byproducts (antigen) in a sample of the patient's blood. Serologic methods have important advantages over direct examination, but require in most cases specialized equipment and reagents and have never achieved wide practical use.

Biotechnology is leading to the development of new diagnostic methods based on the use of monoclonal antibodies (MAbs) and nucleic acid (DNA or RNA) hybridization and promises to revolutionize diagnosis by offering quick, simple, accurate tests when and where they are needed.

The conventional methods of diagnosing infectious diseases and identifying disease-producing organisms include the following:

- the clinical impression of the physician or other health worker (this is the most widely used method of diagnosis);
- direct examination of clinical specimens (e.g., blood, stool, urine, sputum, or tissue biopsy) using light or electron microscopy to identify the disease-producing organism (e.g., malaria parasites, intestinal amebae and helminth eggs, leishmanial organisms, mycobacteria that cause tuberculosis and leprosy, filaria);
- X-ray or computed tomography (CT) scan to image internal pathogens (e.g., amebic abscesses, echinococcal cysts); occasionally useful, but not routine;
- xenodiagnosis, by permitting an insect vector to feed on a patient and then examining the insect by light microscopy to look for the disease-producing organism after it has multiplied to a detectable density (e.g., to diagnose Chagas' disease);
- culture of a specimen from a patient in a test medium, tissue culture, or by inoculation into an animal to allow it to multiply to a detectable density, followed by direct examination for the disease-producing organism, or use of serologic techniques (e.g., for many bacteria and viruses);
- skin tests, demonstrating hypersensitivity reactions to disease antigen (e.g., to diagnose tuberculosis);
- serologic methods to detect antibodies to the pathogenic organism in the patient's serum; and
- demonstration of a pathogen byproduct, such as an antigen by chemical methods (e.g., schistosomes).

Photo credit: Office of Technology Assessment

Xenodiagnosis, by allowing laboratory-raised reduviid bugs to feed on suspected Chagas' disease victim.

CONVENTIONAL DIAGNOSTIC TECHNIQUES

Direct Examination

Laboratory diagnostic methods that directly identify a disease-producing organism by microscopy of clinical samples can provide a definitive diagnosis. Constraints on this approach include the following:

- specially trained personnel are required;
- the work quickly becomes boring and repetitious, while still demanding concentration and attention to detail;
- procedures are relatively time-consuming and require equipment and materials that are often inadequate in endemic countries;
- in some cases, the organisms are not detectable either because:
  - —they are not present in routine clinical samples (e.g., the organisms that cause trichinosis, toxoplasmosis, hydatid disease, hepatic capillariasis, toxocariasis);
  - —they are not detectable in routine clinical samples until a later stage of infection (e.g., filaria); or
  - —their densities are very low, making sensitive diagnosis difficult (e.g., in chronic malaria and chronic Chagas' disease); and
- identifying the disease-producing organism is difficult because some species appear similar (e.g., intestinal amebae, malarial parasites, the African schistosomes with terminally spined eggs, leishmanial organisms).

Serologic Diagnostic Techniques

Serologic diagnosis depends on two types of immunologic methods, which are discussed further below:

- methods for detecting specific antibodies (the immunoglobulins IgG, IgM, IgA, IgE, or IgD) in a sample of the patient's blood; and
- methods involving the use of immunoglobulins for the detection of antigen (the disease-producing organism or its byproducts) in a sample of the patient's blood.

For the detection of antibodies, there are a variety of immunologic methods, all of which can be adjusted by dilution of reagents to give a measure of intensity of reaction (a titer). Because the various immunoglobulins have different roles and develop at different times in an infection, different titers will be obtained at various stages in the infection. (For instance, the immunoglobulin IgM is usually an early but short-lived immune response to infection, whereas IgG production develops more slowly and then continues long after resolution of the infection.) A positive test for antibodies may indicate current infection or the immunity that follows exposure to the infectious agent. In some cases, the test may remain positive for extended periods after the infectious agent has been eliminated by drug treatment.

For the detection of antigen, methods have not been widely developed until recently, for technical reasons. The current advances in MAb technology make antigen detection more feasible.

Serologic Methods for Detection of Antibody

The principal serologic methods for detecting antibodies in a patient's blood are described below.

1. **Complement fixation (CF) test.** This method is well established and has been applied to all viral, bacterial, and parasitic diseases. Complement, a substance normally present in the blood, fixes or binds antigen to antibody. The CF test is performed by adding the known antigen and complement (prepared in the laboratory) to an individual's serum in a test tube. If antibodies are present in the patient's serum, antigen-antibody complexes are formed. No reaction indicates a lack of specific antibody. The CF test requires well-standardized reagents, some of which have limited shelf-life or limited availability in the tropics.

The CF test is widely used for viral diagnosis and is most effective for parasite diagnosis of American trypanosomiasis (Chagas' disease) and schistosomiasis. It is highly specific, but relatively insensitive. It requires large amounts of antigen per test. The CF test is performed adequately only at specialized centers, and it is not considered a test for general, practical use in the tropics (388).

2. **Agglutination assays.** Clumping of particles due to the interaction of antibody and antigen is the basis of all agglutination tests. In most variations, the disease-producing organism or its byproduct (antigen) is fixed to particles, and the particles are added to a test sample that may contain the complementary antibody. If the complementary antibody is present, the particles agglutinate. Each variation of this technique has acquired its own name. The principal agglutination techniques for detection of antibody are described below. (The complementary techniques for detection of antigen are noted in a later section.)

a. **Direct agglutination test.** In the direct agglutination test, whole organisms suspected of causing disease are added to the individual's serum. If antibody is present in the serum, the organisms clump together. The test is simple, but it requires pure, stable antigen preparations (from culture in vivo or in vitro), the test result interpretation is subjective, and there can be complicating autoagglutination reactions that result in false positives.

b. **Indirect hemagglutination (IHA) test.** In IHA tests, the antigen is attached to a carrier, such as specially prepared red blood cells or latex particles. When the individual's serum is added, the particles clump if specific antibody is present. IHA tests have been developed for malaria, Chagas' disease, leishmaniasis, amebiasis, rotavirus, and hydatid disease. IHA tests are simple to perform, can be used to test minute volumes of serum, and can be automated but the end-point reading is subjective. The preparation of test particles is not reproducible, the antigens have short shelf-lives, and there are problems in standardizing antigens from batch to batch.

c. **Hemagglutination inhibition (HI) test.** Various micro-organisms can clump or agglutinate red blood cells from test animals under specified conditions. In the HI test, prepared red blood cells, an appropriate micro-organism (e.g., a virus), and a test serum are combined. If the individual's serum contains antibody specific to that virus, the hemagglutination will be inhibited, because the antibody combines with the organism and cannot react with the red blood cells. The lack of agglutination indicates a positive test.

3. **Neutralization test.** Many viruses damage cultured cells in certain detectable ways. When test serum containing a specific antibody is added to a test well containing the cell culture and the virus (both known quantities prepared in the laboratory), the damage is inhibited, indicating a positive test for the antibody. Neutralizing antibody is usually the first to appear, early in the acute phase of the infection.

4. **Precipitin (or immunodiffusion) test.** With this method, two separate wells are cut into a semisolid substrate such as agar. Serum components are put in one well and antigen in the other. As they migrate, an observable precipitation line is formed where antibody and antigen combine. No precipitation line forms in the absence of antibody.

Counterimmunoelectrophoresis (CIE) and immunoelectrophoresis are adaptations of the precipitin test in which an electric current is passed through the substrate, causing the reactive components to migrate more rapidly and with greater resolution.

The relatively simple diffusion methods are suited to the tropics, but these methods are of limited practical value because of their insensitivity and the long period of time before results. The electrophoretic methods require equipment and a power supply, and the results are complex and somewhat difficult to interpret (98).

5. **Labeled immunodiagnostic reagent assays.** The various types of labeled immunodiagnostic reagent assays all involve a similar procedure. The test antigen is attached to a slide or test well (e.g., blood drops from malaria-parasitized monkeys are dried on microscope slides; in vitro cultured organisms are fixed to plastic test wells). The patients' serum is incubated as a drop on the slide or in the well. If antibody is in the serum, it binds to the fixed antigen. The excess serum is washed off, leaving the antibody sticking to the antigen on the slide. Then a second antibody is added that reacts with any antibody-antigen complexes that

were formed. This second reaction depends on the unusual structure of antibodies—one end of an antibody is a totally unique fit for just one antigen, and the other end is a totally generic molecule. The second lab-prepared antibody has a unique end which recognizes the generic end of other antibodies. This second antibody is also linked to a chemical marker that is detectable—a fluorescent molecule, a radioactive molecule, or an enzyme.

One type of labeled immunodiagnostic assay, the **indirect fluorescent antibody (IFA) test**, uses slides prepared with antigen attached (usually a fixed, cultured organism). After serum antibody binds to the antigen, the second lab-prepared antibody is added to react with any antibody-antigen complexes that were formed. This second antibody is linked to a chemical marker that fluoresces under ultraviolet light. A special microscope is used to detect fluorescence. If the slide fluoresces, it indicates that the original human serum contained the antibodies in question. Microscope slides are easily prepared with antigens stable for long periods for a number of viruses, bacteria, and parasites. Disadvantages of IFA tests are that a well-maintained, carefully calibrated, and expensive fluorescent microscope is needed, the test is time-consuming and somewhat subjective, and with many helminths, there are cross-reactions (388,421)

The **enzyme-linked immunosorbent assay (ELISA)** is complex to describe, yet simple and elegant in its determination. It is rapidly being adapted to the diagnosis of a wide range of organisms. As shown in figure 8-1, the ELISA for the detection of antibody involves attaching specific antigen (a known quantity of test organisms prepared in the laboratory) to a test well or plate, then adding the patient's serum with suspected specific antibody, then adding a lab-prepared second antibody which is linked to an enzyme. The final component added is a chemical substrate whose color changes by the action of the enzyme. The degree of color change is read qualitatively by eye or quantitatively by photometric instrument to give an indication of the amount of antibody present in the serum specimen.

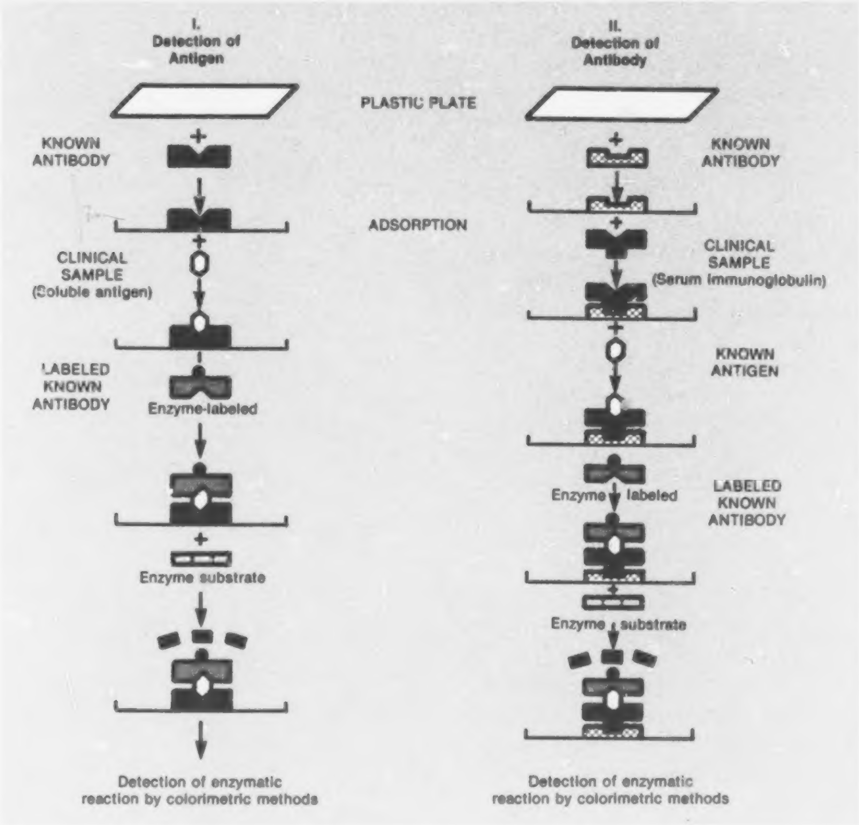
The ELISA uses small sample volumes. Large numbers of specimens can be processed, so the procedure is useful for epidemiologic studies. For developing countries, the ELISA has clear advantages, since it can be done in simple laboratories, and the reagents are stable if refrigerated. Widespread field testing of ELISA procedures is under way for African sleeping sickness and Chagas' disease, leishmaniasis, amebiasis, malaria, filariasis, and schistosomiasis (82,353,389) One great advantage of the ELISA procedure is that the result can often be judged positive or negative by the naked eye. ELISA tests are rapidly being developed and improved.

The **radioimmunoassay (RIA)**, a procedure similar to ELISA, can be carried out using a second antibody labeled with a radioactive compound, which is then read in a scintillation counter. RIA is very sensitive and very quantitatively accurate, but it requires a laboratory equipped to deal with radioactive isotopes, which carry a risk and have a short shelf-life. RIA has been a valuable research tool, but not a practical diagnostic tool (335).

6. **Other tests: circumoval precipitin tests (COPT)** are examples of the variety of serologic tests used for detection of specific pathogens. The COPT for schistosomiasis uses standardized prepared schistosome eggs obtained from animal infections. The eggs are incubated in patient sera, which causes a characteristic precipitate to form around the egg, if antischistosomal antibodies are present.

The **thin-layer immunoassay** for the detection of antibody is depicted in figure 8-2. This technique uses antigen in test plates to capture specific antibody from test serum. The antigen-antibody complexes form a thin layer that attracts water. When the test plate is exposed to water vapor, water visibly condenses on the immune complexes in large droplets which form a pattern distinct from the background. The thin-layer immunoassay has been used to detect the presence of viruses, schistosomes, and amebae. It requires relatively large amounts of antigen, however, and in some cases has low sensitivity (421).

Figure 8-1.—Enzyme-Linked Immunosorbent Assay (ELISA) Technique for Detecting Antigen or Antibody


I. Detection of Antigen
PLASTIC PLATE
KNOWN ANTIBODY
ADSORPTION
CLINICAL SAMPLE (Soluble antigen)
LABELED KNOWN ANTIBODY
Enzyme-labeled
Enzyme substrate
Detection of enzymatic reaction by colorimetric methods
II. Detection of Antibody
KNOWN ANTIBODY
CLINICAL SAMPLE (Serum immunoglobulin)
KNOWN ANTIGEN
Enzyme labeled
LABELED KNOWN ANTIBODY
Enzyme substrate
Detection of enzymatic reaction by colorimetric methods

I. Detection of antigen in a clinical sample:
- Antibody to the antigen being tested for is adsorbed to a plastic plate.
- The clinical sample is added. If the specific antigen is present, antigen-antibody complex forms on the plastic plate.
- A second antibody to the antigen, with a specific enzyme attached, is added to the plate. The enzyme-labeled antibody adheres where antigen-antibody complexes were formed.
- An enzyme substrate is added. If it comes in contact with the enzyme, a reaction occurs that produces a visible color change. If no complexes are formed, there is no reaction.

II. Detection of antibody in a clinical sample:
- The process is the same as the one for detecting antigen, but includes one additional step: known antigen is added, resulting in one more layer in the final complex.

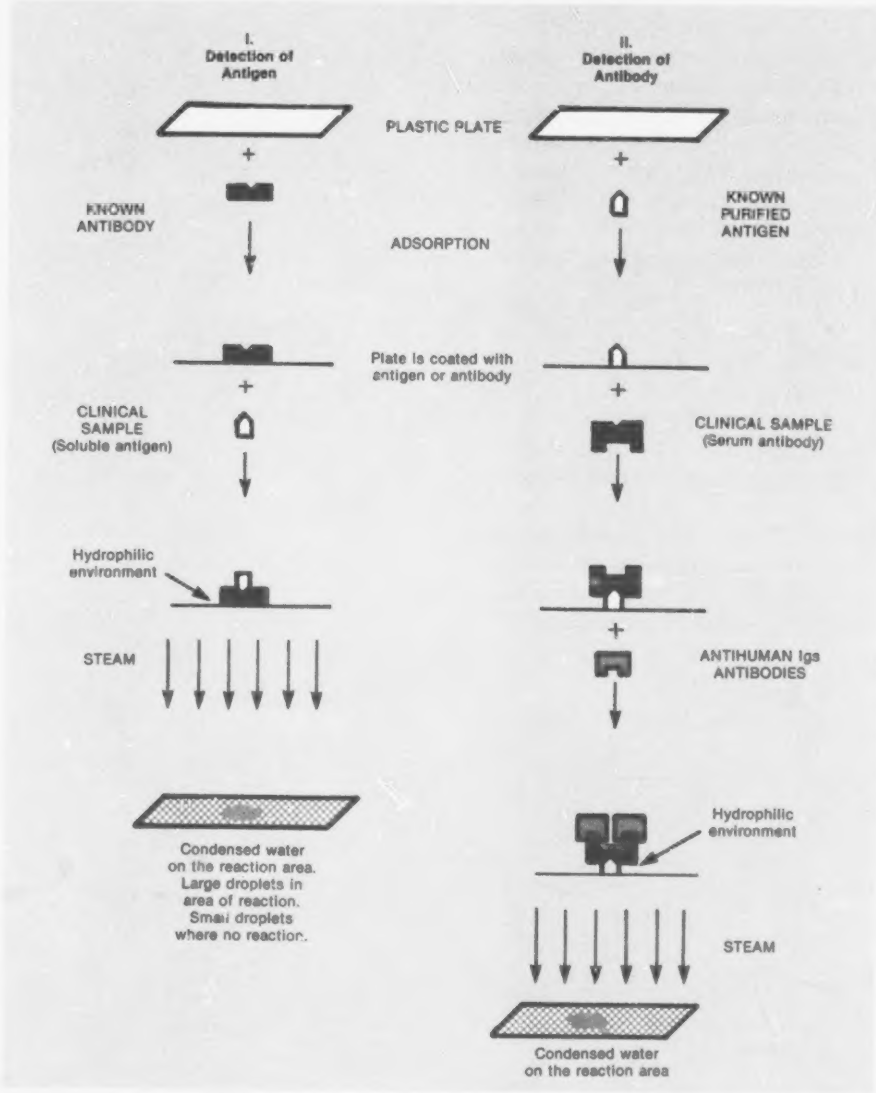
SOURCE: World Health Organization, *Rapid Laboratory Techniques for the Diagnosis of Viral Infections*, Technical Report Series #661 (Geneva: WHO, 1981).

Serologic Methods for Detection of Antigen

The serologic techniques described above focus on antibody detection, which has two inherent disadvantages (388): 1) there is always a delay between infection and development of a detectable level of antibody; and 2) antibody levels persist after clearance of an infection. Thus, false negative and false positive results often occur.

Direct immunologic detection of the antigen itself can be preferable. A serious drawback of this approach is that, compared to the presence of antibodies, antigen presence is a short-lived occur-

Figure 8-2.—Thin-Layer Immunoassay (TIA) Technique for Detecting Antigen or Antibody


I.
Detection of Antigen
II.
Detection of Antibody
PLASTIC PLATE
+
KNOWN ANTIBODY
ADSORPTION
KNOWN PURIFIED ANTIGEN
Plate is coated with antigen or antibody
+
CLINICAL SAMPLE (Soluble antigen)
CLINICAL SAMPLE (Serum antibody)
Hydrophilic environment
STEAM
+
ANTIHUMAN Igs ANTIBODIES
Condensed water on the reaction area. Large droplets in area of reaction. Small droplets where no reaction.
Hydrophilic environment
STEAM
Condensed water on the reaction area

Detection of antigen (I) or antibody (II) in a clinical sample:

- Either known antibody (I) or known antigen (II) is adsorbed onto a plastic plate.
- A clinical sample is added. If the sample contains the appropriate antigen (I) or antibody (II), an antigen-antibody complex is formed on the plastic plate.
- For the detection of antibody (II), a second antibody is added, which is an antibody to the antibody for which the test is done.
- A surface on which an antigen-antibody reaction has taken place is hydrophilic, i.e., it attracts water. The background is hydrophobic, i.e., it repels water. When exposed to steam, large water droplets form in the areas where the reaction has taken place, and small droplets form on the background.

SOURCE: World Health Organization, *Rapid Laboratory Techniques for the Diagnosis of Viral Infections*, Technical Report Series #661 (Geneva: WHO, 1981).

rence, especially for viral diseases. Current serologic methods for detecting antigen are described below.

1. **Labeled immunodiagnostic reagent assays.** The principal methods for detecting antigen are labeled reagent immunoassays that are modifications of the ELISA, RIA, or IFA tests for antibodies (described above) and employ a known specific, lab-prepared *antibody* as the first reagent to adhere to the plastic well. The ELISA technique for the detection of antigen is shown in figure 8-1.

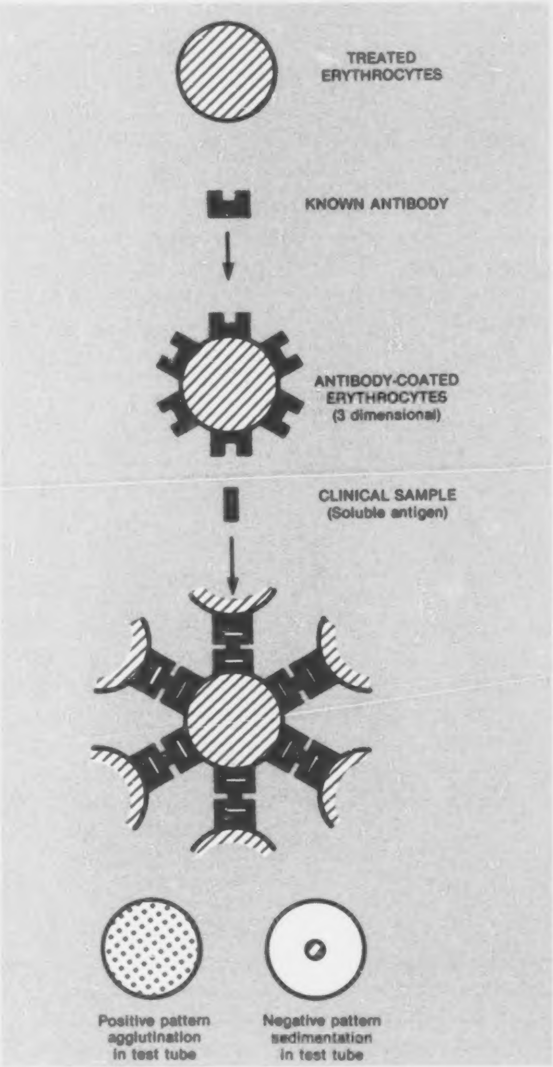
Labeled immunodiagnostic assays for detecting antigens have been applied to viral infections, amebiasis, toxoplasmosis, onchocerciasis (93), schistosomiasis (84), malaria, Chagas' disease, other protozoan infection (8), acute respiratory infectious (ARIs) caused by bacteria, and for detection of certain bacterial toxins causing diarrheal diseases. These tests can identify minute quantities of antigen, but their sensitivity has still been less than the sensitivity of direct examination techniques. As newer methods using MAbs are developed, sensitivity should improve greatly.

2. **Agglutination assays.** As indicated earlier, clumping of particles due to the interaction of antibody and antigen is the basis of all agglutination tests. In the following variations, the disease-producing organism or its byproduct (an antigen) is detected by complementary antibody fixed to particles which are added to a test sample.

In the **coagglutination (COA) test**, a bacterium (*Staphylococcus aureus*) is used as the carrier particle for a specific antibody. When the antibody is mixed with a serum sample, it will agglutinate if the bacteria of interest are present. In a similar test, the **latex agglutination (LA) test**, latex particles are the carriers. Technical considerations determine which method is superior for the diagnosis of any particular agent.

The **reverse passive hemagglutination (RPHA) test**, shown in figure 8-3, is used for rapid detection of viruses (smallpox, arboviruses, and hepatitis B). Here again, specific antibody is fixed to particles that agglutinate if the appropriate antigen is present in the test serum.

Figure 8-3.—Reverse Passive Hemagglutination (RPHA) Technique for Detecting Viruses


TREATED ERYTHROCYTES
KNOWN ANTIBODY
ANTIBODY-COATED ERYTHROCYTES (3 dimensional)
CLINICAL SAMPLE (Soluble antigen)
Positive pattern agglutination in test tube
Negative pattern sedimentation in test tube

Detection of viruses in a clinical sample:

- Known antibody is added to and adheres to the surface of treated erythrocytes (red blood cells).
- A clinical sample, suspected of containing the specific virus, is added. If the virus is present, complexes are formed linking antibody-coated erythrocytes together, visible in the test tube as an agglutination pattern.

SOURCE: World Health Organization, *Rapid Laboratory Techniques for the Diagnosis of Viral Infections*, Technical Report #661 (Geneva: WHO, 1981).

Constraints on Serologic Diagnosis

Serologic diagnostic techniques provide some attractive technical options. Such methods can be very effective in epidemiologic surveys (203). Some of these methods have been adapted for field use in tropical areas (e.g., by absorbing a few drops of blood onto a filter paper strip for later examination in the laboratory). Serologic methods also are more easily automated than techniques of direct examination and thus have increased precision. Despite these features, however, very few such immunologic methods have proved to be of practical value in routine diagnostic laboratory practice. Most have remained in the research laboratory (388).

Several constraints limit the usefulness of serologic techniques in medical practice or for public health measures:

- Serologic procedures require sophisticated laboratory instruments and equipment. In many developing countries, basic services such as transport, electricity, and water are inadequate or unreliable, and trained personnel are lacking.
- In industrialized countries, where the expensive, sophisticated tests are feasible, few centers specializing in "exotic" diseases have the demand to justify doing the procedures. The result is that most medical facilities rely on the conventional techniques of direct examination.
- Parasite antigens are highly cross-reactive. Cross-reactivity, coupled with the presence commonly of more than one organism in an individual, makes the interpretation of serologic tests difficult.
- Test reagents vary widely, and different laboratories can report grossly divergent results using the same batch of antiserum. The need for standardization and for high-quality antigens is acute (177,388).
- "Paired sera" (from patients in the acute and convalescent stages of illness) may be required if changing antibody titer is a criterion for diagnosis.
- Many individuals are reluctant to have blood drawn.

Many of the antigens needed for serodiagnostic tests can be cultured in vitro. In vitro cultivation techniques are improving rapidly for many of the agents of tropical diseases (296). The other major source of precisely defined antigens for diagnosis will be recombinant DNA technology.

GENETIC TOOLS FOR IMPROVING DIAGNOSTIC TECHNIQUES

It is one thing for a university medical center to undertake the diagnosis of a single patient recently returned from the tropics and quite another for a survey team to apply diagnostic methods under field conditions to large numbers of local people in isolated, endemic, tropical areas. For use in the field, there is a great need for simple, reproducible, and inexpensive diagnostic methods that can provide a clear-cut indication of the status of an infection, and a specific identification of the causative agent. This need is especially acute because of the current work on developing vaccines for a variety of tropical diseases (see ch. 7). Without a means for accurate determination (or at least reliable estimation) of the prior exposure and immune status of vaccinees and controls, and their post-inoculation followup, valid field testing of these vaccines will not be possible.

Biotechnology (see ch. 5) is leading to the development of new diagnostic methods based on the use of MAbs and recombinant DNA (or recombinant RNA) techniques. These techniques, which are advancing rapidly, hold out a promise of accuracy and simplicity. In addition, they generally require smaller amounts of clinical samples than have heretofore been necessary. DNA and RNA hybridization tests hold great promise, but further development and testing are necessary before many of the tests can achieve widespread use in the field (137).

Monoclonal Antibodies (MAbs)

Immunodiagnosis using MAbs is building on the past 20 years of research in serologic diagnostics. The revolution is in making these diagnostic test procedures much more sensitive and specific, more reproducible, faster, and more economical. Three diagnostic uses of MAbs are being developed (84):

- immunoassays of high specificity, i.e., making existing serologic diagnostic tests better by production of purer reagents;
- "two-site" immunoassays of antigen (the pathogenic organism itself or its byproducts) from appropriate body fluids, using two monoclonals directed against different binding sites of one antigen—one MAb to catch the antigen and another to label it for detection; and
- the use of cross-reactive MAbs to absorb certain common antigens out of crude mixtures, making possible the sensitive and specific detection of an antigen of interest.

MAbs have been used to distinguish between organisms that cross-react in conventional serologic tests, e.g., *Trypanosoma cruzi* and *Leishmania braziliensis* (71); between closely related species of New World *Leishmania* (283); between strains within single species of *Theileria* (277) and *Leishmania* (145); between genetic variants (43) or life cycle stages (111) of *Trypanosoma rhodesiense*; and between larval and adult *Schistosoma mansoni* (332).

Assays of antigen in which MAbs are affixed to cellulose fibers or other solid substrates (e.g., plastic test wells) have been referred to as "dipstick technology" (136). Antigen present in blood, urine, stool, etc., binds to the MAbs, and the combination is detected by a second antibody through an ELISA-like color reaction. Immunodiagnosis using MAbs as probes for parasite antigen has been applied to such diverse conditions as tuberculosis (153), hydatid and other larval tapeworm diseases (78,79), Chagas' disease (9), onchocerciasis (93), and schistosomiasis (83,84,239). ELISA procedures using MAbs have been described for rotavirus infection (424), toxoplasmosis (8), and schistosomiasis (240). MAbs have been used to identify malarial organisms within mosquitoes without the need for exhaustive dissection and microscopy.

Nucleic Acid Hybridization Probes

The use of a nucleic acid (DNA or RNA) hybridization probe to identify the actual DNA or RNA of a disease-producing organism (112) is a promising diagnostic technique that has some important advantages, perhaps most important of which is high specificity. Scientists are now able to isolate and then reproduce ("clone") DNA segments from known organisms, label the segments radioactively, separate them from the normal double-stranded state to a single-strand state, and then test them against unknown specimens of DNA (e.g., in a stool sample) for the ability to hybridize (re-form double-stranded DNA). If the suspected organism is present, then the labeled DNA and the sample DNA, being the same, will hybridize. The radioactivity or fluorescence or enzymatic color change can then be detected.

The nucleic acid hybridization techniques for different organisms vary, but such techniques can be developed to be simple, practical, and relatively inexpensive. Using these techniques, investigators can screen large numbers of samples at one time. Samples can be collected and stored for several weeks before the test is completed. Nucleic acid probes should be useful for large-scale epidemiologic and surveillance studies (117,246). The major disadvantages of the tests that use radioactive labels are the radiation hazard and the short shelf-life of the radiolabeled reagents. These problems can probably be circumvented in most cases by substituting tests that use color change methods in place of radiolabels (196,202).

The uses of nucleic acid hybridization techniques to detect malaria parasites (122), *Leishmania* spp. (409), *Escherichia coli* (246), and rotavirus, and to detect *Salmonella* bacteria in food products (116) are described below. The utility of nucleic acid hybridization probes in the field in developing countries must be further evaluated, but good results have been reported in detection of rotavirus and *E. coli* in stool sample dots. Although nucleic acid hybridization is still in its early development phase, it may well progress to a predominant role in diagnosis.

DIAGNOSIS: CURRENT STATUS FOR SELECTED TROPICAL DISEASES

Malaria

Since the emergence of drug-resistant malaria parasites in the late 1950s, the importance of specific diagnosis, including a determination of whether the malaria parasites are drug resistant, has been greatly elevated. Previously cheap, safe, effective, and completely standardized antimalarial treatment regimens were available, and, presumptive treatment, without definitive diagnosis, was freely administered. Now, however, appropriate treatment rests on accurate diagnosis.

Conventional Diagnosis

Diagnosis of malaria based on physical examination alone can be difficult, because malaria's symptoms are protean. For that reason, laboratory diagnosis of malaria is important. The standard method of diagnosis is by microscope examination of a stained blood smear made from a finger-prick. The presence of malaria parasites is definitive. Under field conditions, there are generally quite a few false negatives, because people with malaria do not always have large numbers of malaria parasites circulating in their blood. In the absence of microscopically confirmed infection, a presumptive diagnosis of malaria may still be made, based on clinical symptoms. Treatment is given to reduce the parasite load of the population and, in effect, to prevent mosquitoes from acquiring malaria.

Although methods for the serologic diagnosis of malaria have long been available (CF, IHA, and IFA tests), they are not widely used for two reasons. First, the need for equipment and materials to perform the tests cannot always be met. Second, the tests demonstrate the presence of antibodies, which persist after cure and may indicate previous infection. In an endemic country where infection is always possible, such tests do not greatly help the decision to treat. Nevertheless, these tests have been useful for epidemiologic surveys (especially IHA and IFA tests (168)), and for special purposes such as establishing the presence of antibody in a particular patient in whom infection is suspected but cannot be demonstrated directly (e.g., in a blood donor who is suspected of having transmitted malaria to a blood recipient).

Recent Progress

Serologic Diagnosis.—Improved serologic methods for the detection of blood stage malarial antigens are being developed: RIA and ELISA tests for the detection of parasitized red blood cells have been developed. Sensitivity of parasite detection with these tests is encouraging, and reproducibility should improve as standard reagents become available (353).

In the hope of establishing standard reagents for malaria serology, the World Health Organization's (WHO) Immunology Research and Training Center, Geneva, has established a registry of malarial MAbs collected from other laboratories and evaluated for potential value as serodiagnostic reagents.

Sporozoite Diagnosis.—Immunologic work on plasmodial sporozoites has led to the development of two methods to test for sporozoites in mosquitoes. Such testing has importance for epidemiologic studies in determining the degree to which various mosquitoes function as vectors of species of malaria parasites.

In one method, MAbs are used to identify sporozoites in mosquito squashes by a direct binding assay, which is species-specific. This method was developed as an RIA, but an ELISA procedure is being developed as well. Although the test procedure must be carried out in a centralized laboratory, the samples are stable without refrigeration and easily handled, and the results are available within an acceptable time interval for epidemiologic purposes.

A second method, another new immunoradiometric test (inhibition of idiotype-anti-idiotype interaction, or "4 i-assay"), has been developed to detect circumsporozoite protein (280) and is based on inhibition of binding of two MAbs. The first MAb is against malarial antigen, and the second MAb is against the first MAb (thus called an idiotype, which resembles the original antigen). When both MAbs are mixed in a test well, they bind together in a detectable way, unless the test sample contains antigen. If the sample contains antigen, the antigen will bind specific antibody, thus inhibiting the two MAbs from interacting (inhibition indicates the presence of true parasite antigen). This test is sensitive enough to distinguish between different species of malaria parasites. The method has general applicability. Furthermore, because the two immunoglobulin reagents are MAbs, this method does not require cultivation and purification of antigen from parasite, and the two immunoglobulin reagents are completely pure (422).

DNA Hybridization.—Work on development of a rapid diagnostic test using specific DNA hybridization is proceeding. A recent publication describes experimental success in identifying *Plasmodium falciparum* parasites in samples of blood from in vitro culture and from malaria patients (122). This method is still at a very preliminary stage in relation to any practical use, because the sensitivity is no better than microscopic examination of a stained blood film, and the procedure involves a number of laboratory steps that take about 24 hours to complete.

In Vitro Cultivation of Malaria Parasites and Microtest of Drug Sensitivity.—With the current situation of widespread resistance to drugs by malaria parasites, diagnosis of drug susceptibility in parasite isolates is an important epidemiologic task. The method for in vitro cultivation of *P. falciparum*, the malaria species with widespread drug resistance, has been adapted to several techniques for testing drug susceptibility against the primary antimalarials. The tests are available in kit form from WHO.

Research Needs

Greater standardization of serologic tests for the detection of malaria antigens and antibodies is needed. These tests need to be adapted for field applications, both as quick and easy diagnostics at remote or poorly equipped treatment posts and to assess any vaccination trials that may be attempted in the future. Assessment of vaccination trials will rely on detecting antibodies in individuals who did not have antibodies before vaccination as well as determining infection rates post-vaccination.

A quick and easy field method is needed to establish the existence of infection (to conserve drug for true cases) and to differentiate species of *Plasmodium* (for appropriate drug type). Similarly, a field method to detect infection in mosquito vectors is needed to assess the impact of vaccination on the overall risk of transmission.

A less urgent but important need is a method of screening blood bank donations to prevent transfusion malaria.

Schistosomiasis

The major schistosomes infecting humans are *Schistosoma mansoni*, *S. japonicum*, and *S. haematobium*. Current control for schistosomiasis calls for the identification and treatment of all infected persons at regular intervals (usually 6 months) (353). Quick and reliable diagnosis is essential to identify people infected and for assessing the effectiveness of the treatment. To determine optimal treatment, it is necessary not only to diagnose the presence of infection but also to determine how heavy the parasite load is.

Conventional Diagnosis

Direct examination of feces or urine for characteristic eggs is the classical method of diagnosis of schistosomiasis. There are a number of methods for concentrating the sample to maximize the chance of detection and a number of methods for accurately estimating the number of eggs in order to estimate the parasite burden in the host. Cytoscopy or sigmoidoscopy (viewing through instruments inserted into the body) is occasionally used to detect lesions, and rectal biopsy is sometimes used. There is a CF test, an intradermal skin test, and the COPT to help confirm diagnosis.

Recent Progress

Two new techniques have been developed for *S. haematobium* diagnosis. One is the filtration of urine using a reusable plastic woven filter which isolates excreted eggs. The second is a prototype image processing and pattern recognition apparatus for automated *S. haematobium* egg counts that has been tested in the laboratory and is undergoing field trials in endemic areas (353).

A new diagnostic kit has recently been made available by the Program for Appropriate Technology in Health, a nonprofit, nongovernmental organization. The kit is for the diagnosis of *S. haematobium*, and it is designed for quick, practical, and reliable field use.

In a special collaborative study sponsored jointly by WHO and the Edna McConnell Clark Foundation, eight research laboratories evaluated a number of procedures for immunodiagnosis of schistosomiasis (247). Included were the COPT, ELISA, IHA, IFA, RIA, and other procedures, testing a pool of banked sera against a variety of schistosomal antigens. A study of this type is of great value in developing and standardizing materials and methods for immunodiagnosis.

Several candidate antigens for immunodiagnosis have been identified (59). There are several modifications of the ELISA procedure, including an inhibition-ELISA used to detect and characterize schistosomal antigens (1). Many laboratories have developed MAbs for identification of relevant antigens or for species diagnosis (e.g., 1,83,84,99,237,238,239,240).

Many workers are attempting to identify and isolate relevant protective antigens, and at least half of those are actively engaged in gene cloning experiments. Several laboratories are devising improved diagnostic methods, primarily with ELISA-based tests. Most schistosomiasis experts believe that effective MAb-based diagnostic tools are very near or within 5 years of introduction (16).

Research Needs

Field methods for quick and easy diagnosis of schistosomiasis are being introduced but still require evaluation and standardization.

Trypanosomiasis

African Sleeping Sickness (African Trypanosomiasis)

There are two forms of African sleeping sickness in humans. *Trypanosoma brucei gambiense* causes the chronic form found in west Africa. *T. b. rhodesiense* causes the acute form in east Africa and also infects livestock over large areas of the continent.

Conventional Diagnosis.—Direct examination for African trypanosomes is done from stained blood preparations, but parasites are extremely difficult to find. In the chronic form (*T. b. gambiense*), fluid drawn by needle from lymph nodes in the neck is examined. The number of parasites detected varies daily. In the acute form (*T. b. rhodesiense*) too, parasites may vary in density, making diagnosis difficult. Cerebrospinal fluid may also be examined. Inoculation of laboratory animals or culture on appropriate media to allow the parasites to multiply is sometimes useful.

Immunodiagnostic techniques have been available for many years (IFA and CF tests), but these must be performed in central laboratories. The ELISA has also been found effective in the laboratory but not for the field (353). All of these tests are valuable for epidemiologic studies, but the delay in reaching a diagnosis limits their usefulness for patient care.

Recent Progress.—Tests for antibodies against *T. b. gambiense*, which can be read within minutes and carried out with blood obtained from a finger-prick, are under development and evaluation in the field. They are the card agglutination test for trypanosomiasis (CATT), the Cellognost test (a commercial technique based on indirect haemagglutination), and the Tryptest. CATT is being evaluated on a large scale in west Africa. This test has been found to be as specific as the IFA test. It is easily transported to the field, requires little technical skill, and gives results within minutes. The lack of stability of the antigen and storage under field conditions are problems to be worked on. Antigens to diagnose *T. b. rhodesiense* are being sought (353).

Two other techniques are also being evaluated: the miniature anion exchange column technique and the microhaematocrit buffy coat centrifugation method. Both have shown greater sensitivity than blood film examination (208). Development of the double centrifugation technique for the detection of trypanosomes in cerebrospinal fluid has improved diagnosis of central nervous system involvement (353).

The three subspecies of the *Trypanosoma brucei* species complex are morphologically indistinguishable. Two species (*T. b. rhodesiense* and *T. b. gambiense*) are infective to humans, causing sleeping sickness; the third (*T. b. brucei*) is infective to wild and domestic animals but not to humans. Scientists' inability to distinguish between human and animal forms has epidemiologic importance for determining risk to humans where animals are found infected. Culturing of trypanosomes in human serum is widely used for determination of infectivity to humans. Isoenzyme electrophoresis, DNA hybridization, and comparative IFA with standardized sera have also been used recently.

Research Needs.—Several avenues of research may be nearing fruition for the diagnosis of African trypanosomiasis. There is a need to develop sensitive and specific techniques suitable for field use.

Chagas' Disease (American Trypanosomiasis)

Chagas' disease, caused by the protozoan parasite *Trypanosoma cruzi*, affects more than 12 million people in Latin America (229). Acute and chronic phases of the disease vary somewhat from one region to another (166). Most damage is done by tiny, nonflagellated forms within the cells of heart muscle and certain nerve ganglia.

Conventional Diagnosis.—The clinical picture of Chagas' disease is variable. Examination of blood during the acute phase of the disease may reveal parasites. Because of commonly low parasite density in blood, several culture techniques are used to allow the parasite to multiply to a detectable level: inoculation into laboratory animals followed by periodic examination over 60 days; in vitro culture of blood; xenodiagnosis (allowing clean, uninfected reduviid bugs to feed on the suspected patient and then examining the hindgut of the bug for trypanosomes after 2 weeks). All these methods often require repeated attempts.

A CF test (the Machado-Guerreiro test) using *T. cruzi* antigen is available, as is other serologic diagnosis for the direct detection of antibody. Conventional serologic tests for Chagas' disease cross-react with leishmaniasis, leprosy, and syphilis antigens and are not sufficiently sensitive to detect Chagas' disease with assurance. A number of groups are working on development of an immunodiagnostic reagent, and optimism is generally high. Immunodiagnosis of specific antibody is available as a procedure in centralized laboratories, but standardization between laboratories is a problem.

Recent Progress.—Standardization of serodiagnostic techniques has been promoted by the Special Program for Research and Training in Tropical Diseases (TDR). A serum reference bank is now providing standardized lyophilized (freeze-dried) serum samples to laboratories throughout Latin America, and a network of collaborative laboratories has developed protocols for the standardization of reagents, techniques, and procedures (353).

Many workers have developed MAbs that may be useful for the detection of various trypanosome antigens (9,259). Development of ELISA diagnosis is under way in a number of laboratories. Investigators have shown that circulating antigens in acute infections can be detected (7).

A specific diagnostic test is being evaluated using purified cell membrane antigens of *T. cruzi* which are fixed on polyamide strips. After exposure to suspected serum, the strips are treated as in ELISA-type tests to detect any antigen-antibody complexes that would form if the serum were from a person with Chagas' disease. Preliminary results indicate that the test greatly reduces, but does not eliminate, nonspecific reactions.

Blood-transfusion-transmitted Chagas' disease in Brazilian hospitals is a serious problem, and better screening methods for donated blood are needed. An agglutination test for rapid screening of donated blood for *T. cruzi* infection is undergoing evaluation in Brazil, where it was developed, and in a network of collaborating laboratories (353).

A DNA-DNA hybridization probe shows promise of detecting the presence of *T. cruzi* organisms or DNA fragments present in the blood. *T. cruzi*, has a unique, highly variable type of DNA (kinetoplast DNA) that can be used to distinguish species and strains within species. A technique for isolating DNA, cutting it into pieces, and then separating the DNA into recognizable patterns (restriction endonuclease finger-printing of kinetoplast DNA) promises to provide a valuable new tool for epidemiologic and clinical purposes. New subdivisions of *T. cruzi* strains can be demonstrated using this method. These refinements in the taxonomy of the parasites may help to explain the variability of the disease in different localities, including the variation in clinical symptoms and the variable susceptibility or resistance in hosts (353).

Research Needs.—A quick, easy, and reliable method is needed for diagnosis of acute Chagas' disease (when treatment might be prophylactic, and because the treatment is toxic). A means to predict prognosis in different geographic areas also is desirable.

A sensitive test for screening donated blood is also particularly important in Chagas' disease, as infection by transfusion is a serious problem in endemic areas.

Leishmaniasis

Leishmaniasis is a disease with three clinical presentations depending on the leishmanial parasite species. Cutaneous leishmaniasis, caused by either *L. tropica*, *L. mexicana*, or *L. brazilienis* (depending on geographical location), is a self-limiting and usually self-resolving sore at the point of infection. Mucocutaneous leishmaniasis, caused by *L. brazilienis*, begins as a sore but commonly metastasizes and proliferates in the nasal and pharyngeal mucous membranes. Visceral leishmaniasis, or "kala-azar," is caused by *L. donovani* and affects the spleen, liver, bone marrow, and lymph glands.

Conventional Diagnosis

Diagnosis of leishmanial organisms is complicated by the rather uniform appearance of different species under the light microscope, and cross-reactivity of different species with conventional serologic diagnosis. Until recently, microscopic identification and conventional serologic techniques were the only techniques available for diagnosing leishmaniasis.

Photo credit: Office of Technology Assessment

Leishmanial organisms as seen through a light microscope.

The inability to distinguish correctly between species of *Leishmania* can have serious consequences for patients. For example, the lesions of *L. mexicana* and *L. braziliensis* are very similar at first appearance, and both species overlap in many parts of South America. Without treatment, *L. mexicana* is self-resolving, but *L. braziliensis* progresses to gross destruction of the nose and throat. Thus, treatment and followup for *L. braziliensis* infection are critical. But the treatment is itself highly toxic and clearly not to be used for those not requiring it.

Recent Progress

Investigators have developed a DNA hybridization probe to distinguish between *L. mexicana* and *L. braziliensis* (409) using kinetoplast DNA, a unique form of DNA in *Leishmania* and *Trypanosoma* species. Kinetoplast DNA is extracted from growing cultures of various species of *Leishmania* organisms, processed and labeled radioactively. Test material is collected from the patient as "touch preparations" on nitrocellulose filter paper from suspected lesions. Hybridization and analysis are then carried out. Kinetoplast DNA hybridization is highly species-specific and provides a relatively rapid means of diagnosis direct from infected tissue. This has now been tested for the diagnosis of human patients and shows promise.

Another test, called the "DOT-ELISA" test, has been developed, and represents a major advance in the rapid field diagnosis of visceral leishmaniasis. It also is useful for field surveys for identifying infected vectors (267).

In recent years, scientists have prepared MAbs against a variety of antigenic determinants in *Leishmania* species (71,91,139,145,171) and used them to probe for specific morphologic and taxonomic differences. Among the many monoclonals produced, some recognize antigens common to all kinetoplastid (*Leishmania* and *Trypanosoma*) species tested; others bind only to certain strains within a single species. The process of sorting out these specificities and defining the precise nature of the reactive leishmanial antigens should produce advances in knowledge and diagnosis of these organisms.

Research Needs

The diagnosis of *Leishmania* species is very important, because different species produce similar lesions but have very different long-term consequences. DNA hybridization looks promising but needs development for practical use.

In the clinical-epidemiologic area, there are many strains and types of *Leishmania*, but a lack of a good classification system. More field surveys to determine prevalence of disease are needed, and those surveys will require practical field tests.

Filariasis

Filariasis is a collective term for several distinct parasitic infections by insect-transmitted, tissue-dwelling nematodes. The principal species are *Wuchereria bancrofti* and *Brugia malayi*, which cause filarial elephantiasis; and *Onchocerca volvulus*, the cause of onchercerciasis (river blindness) in west Africa, also found in Central and South America.

Conventional Diagnosis

Conventional diagnosis of filarial infections has severe limitations. For lymphatic filariasis (*Wuchereria* and *Brugia*), diagnosis is made by microscope examination of stained blood films to find microfilariae. Density of microfilariae is usually very low, especially during the early stages of infection; and for several species, it is cyclical according to a circadian rhythm, so at times there may be no microfilariae present in the blood. Diagnosis of onchocerciasis (in which the subcutaneous tissues are infected) is made by using a special surgical punch to take tiny snips of skin and then examining this skin under the microscope. Because of cross-reactions with other organisms, no dependable serologic diagnostics for filarial infections are available.

Photo credit: H. W. Brown and F. A. Neva, "Basic Clinical Parasitology," Appleton-Century-Crofts. Reprinted by permission.

Microfilariae of *Onchocerca volvulus* were found in skin snips from these nodules.

Recent Progress

A number of MAbs to filarial antigens have been produced, but immunodiagnosis has been hindered by a high level of cross-reactivity with other helminth antigens. Excretory-secretory antigens, which are released by the parasite and circulate in the host's blood, and surface antigens on the parasite itself are being assessed for use in immunodiagnosis of filaria. The demonstration of circulating antigens in *Onchocerca* infections has made a diagnostic MAb feasible. A good correlation between circulating antigen and presence of parasites was found in one study, using a MAb in an RIA (93). However, the false positive rate was high, suggesting that further refinement of this test is needed. At a workshop held in 1983, 26 researchers from around the world brought about 50 antifilarial MAbs representing the total successful effort to that date (16).

Tests for detection of specific antibodies against filarial worms, for circulating parasite antigen, and for parasite surface antigen are being developed. There is optimism about the possibility of a breakthrough in filarial detection within the next few years. At least one Federal Government laboratory, one in industry, and one in a university are conducting or planning recombinant DNA work for expression of such filarial antigens.

A problem in diagnosing filariasis is the identification of parasites found in wild-caught vector insects. The problem of confusing intermediate life stages of disease-producing organisms with other organisms of little public health significance is common to vector-borne diseases, and represents a special problem in diagnosis which is amenable to solution by modern methods. In areas of west Africa with active vector control programs against onchocerciasis, new insects with potential vectorial capacity are entering controlled areas from adjoining regions. Some of these insects are naturally infected with filarial larvae, possibly parasites of wildlife, that cannot be distinguished by direct examination from larval *Onchocerca*. The development of probes, either by MAbs or possibly DNA hybridization, would permit identification of these nematode larvae and determination of whether they pose a threat to humans.

Research Needs

Very generally, improvements in all aspects of the diagnosis of filarial infections are needed. Because much remains to be learned about the epidemiology and pathology of filariasis, useful diagnostic tests are needed. It is important both for patient care and for research purposes to be able to distinguish animal and human filariae, a task which at present is not always possible.

Leprosy (Hansen's Disease)

Leprosy, caused by the bacillus *Mycobacterium leprae*, is the only bacterial infection among the six diseases targeted by TDR. Since the 1950s, the WHO-recommended strategy of leprosy control through early case finding, followup of contacts, and chemotherapy of patients has proved to be difficult to implement and sustain in many countries (353).

Conventional Diagnosis

The initial diagnosis of leprosy is through recognition of areas of skin that lack feeling (anesthesia) and may be discolored or slightly raised. Definitive diagnosis is made by acid-fast staining and microscope examination of skin biopsy smears from these suspected lesions. This technique is useful in all forms of leprosy, but there may be few bacteria in milder cases, making detection uncertain.

The lepromin or Mitsuda test, a skin test similar to the tuberculin skin test (see below), is used as a prognostic test after leprosy is diagnosed. It tells where the patient is along the immunopathologic scale of disease. A crude suspension of killed bacilli (derived from human leprosy patients) is injected under the skin of the patient. About 3 weeks later, the skin reaction is assessed, allowing a prognosis to guide treatment.

A lymphocyte transformation test has been available for a decade as an indicator of *M. leprae* infection and the potential course of the disease (133). This test is an indicator of cell-mediated immune response. Despite its technical difficulties and subjective interpretation, the lymphocyte transformation test has given useful results in experimental field studies of exposure to leprosy antigen, though it has not become a practical method for routine use. Another test of the cell-mediated immune response, macrophage migration inhibition, is also not used routinely (168). It also possible to isolate and diagnose leprosy by inoculation of a clinical specimen from a suspected case into the footpad of a mouse.

Recent Progress

A phenolic glycolipid molecule has recently been isolated and identified as a unique and specific antigen that is abundant on *M. leprae* and in the skin lesions of leprosy patients. This antigen has been used in an ELISA and has proved to be specific for antileprosy antibody (56). It has been tested experimentally to detect antibody in the blood of leprosy patients, with excellent results (433). This antigen has the potential to be used in a specific diagnostic test for leprosy. Investigators have found that contacts of known cases are more likely than other people to develop antibodies to this antigen. The phenolic glycolipid test may therefore be useful in screening persons at increased risk of developing clinical disease.

A number of apparently *M. leprae*-specific antigens (other than the phenolic glycolipid antigen) have been reported (30,41,149), and MAbs have been produced, which may also prove useful as diagnostics. Serologic tests using IFA, ELISA, or RIA are being developed for epidemiologic studies. The fluorescent leprosy antibody absorption test has shown high specificity and is being evaluated for predictive value in long-term epidemiologic studies (2). Skin tests have been developed for monitoring delayed-type hypersensitivity reactions to *M. leprae* after immunization, though the current test antigens are crude extracts that lack specificity. Other skin test preparations using soluble antigens are being evaluated in the field for predictive value (224,232,346).

Research Needs

A method of culturing *M. leprae* in vitro is an urgent need. Such a method could lead to improved methods for early diagnosis, which in turn would lead to earlier treatment and favorable prognosis. Practical techniques to diagnose leprosy for epidemiologic studies need to be developed to allow better understanding of transmission and susceptibility.

Progress in development of serologic tests that are sensitive and specific has raised optimism about prospects for achieving practical diagnostic techniques for leprosy. The phenolic glycolipid antigen is most promising at present and may be developed into a useful diagnostic test. At present, the main source of this antigen and others is from *M. leprae* grown in armadillos. Obtaining antigens from *M. leprae* grown in armadillos is a slow process. It may be necessary or useful for diagnosis to make important molecules from *M. leprae* using recombinant DNA.

A method for the differentiation of patients with lepromatous leprosy (the severe form with poor prognosis) and tuberculoid leprosy (the mild form with good prognosis) is needed to improve understanding of the epidemiology of the disease and for the early recognition of individuals at high risk of developing disease. The evidence for a genetic basis to resistance needs further study.

Tuberculosis

Tuberculosis remains a major threat to health in many parts of the world, causing several million deaths annually. Tuberculosis control programs are built around vaccination coupled with early case detection, treatment, and followup of active cases. Diagnostic methods and early diagnosis before symptoms are overt are critical to the control of this disease.

Conventional Diagnosis

The consideration of clinical symptoms and examination of direct sputum smears for the presence of the causative tubercle bacilli are the conventional means of diagnosing tuberculosis. If no bacilli are found in a direct smear but tuberculosis still is suspected, culture isolation of bacteria from a clinical specimen (usually sputum, though urine, spinal fluid, or tissue biopsy may be appropriate) is attempted. Culturing also is used to confirm a diagnosis made by a direct smear. Isolation of bacilli from culture is the preferred method, but several serious problems arise: the need to decontaminate the specimen to prevent overgrowth by contaminating bacteria from the oral cavity; the need to collect and culture multiple samples from a suspected case; and the slow multiplication of the bacilli, which means that it may take 3 to 6 weeks for growth to appear.

Chest X-rays continue to be of great value in the diagnosis of tuberculosis, particularly in areas where other diseases (like histoplasmosis) with similar X-ray appearance are absent. X-rays are particularly useful for determining (by noting differences between earlier and later films) that a dormant case has been reactivated.

Microscope examination of stained sputum smears can be used to quickly identify tuberculosis-like bacteria, but does not distinguish virulent tubercle bacilli from look-alikes. In areas of the world where active pulmonary tuberculosis is common, a presumptive diagnosis can be made on the basis of numerous bacteria with particular staining qualities (acid-fast) in the sputum. It is clear, however, that many different species of *Mycobacterium*, in addition to *M. tuberculosis*, can infect humans and cause tuberculosis-like disease. Such environmental mycobacteria (called "atypical mycobacteria") are diagnosed by their characteristics in culture, and cannot be distinguished on direct sputum examination. Some of these (the *M. avium-intracellulare* complex) are associated with acquired immunodeficiency syndrome (commonly known as "AIDS").

The tuberculin skin test is used to identify people infected with tubercle bacilli, by means of an allergic reaction to tuberculosis antigens (delayed-type hypersensitivity). A small amount of tuberculoprotein is introduced into the skin, and the person is observed for an inflammatory reaction 2 to 3 days later. Old tuberculin (a crude concentrate of tubercle bacilli in culture medium) has been replaced by purified protein derivative (PPD), a purer, more standard material.

Three techniques can be used to introduce the tuberculin: 1) the Mantoux test, in which PPD is injected intradermally; 2) the Vollmer patch test, in which tuberculin is applied to the skin on a gauze adhesive strip; and 3) the Tine test, in which the tuberculin is dried on the points of a standard puncture device that is pressed into the skin.

All of the tests have advantages and disadvantages. None of these tests can be used to test anyone already vaccinated against tuberculosis (a large percentage of the population in many developing countries and in a number of developed countries), because vaccinated individuals should react to the challenge. The Mantoux is the most sensitive and reliable test, because a standard volume and amount of tuberculin is injected directly into the skin; however, the procedure of injection by hypodermic is a practical disadvantage. The Tine test is a rapid and easy test for use in large population groups, but it gives a relatively high number of "false positives" (people who test positive but who actually are not infected); positives must be followed up by a Mantoux test. The Vollmer patch test is useful for skin-testing infants and children, but it is less sensitive than the Mantoux test.

A positive tuberculin skin test may be caused by infection with other species of mycobacteria or as a result of previous vaccination. A negative tuberculin test is strong evidence against the tuberculosis diagnosis, but can also result from loss of potency in stored PPD, and is occasionally observed in far advanced cases.

Recent Progress

ELISAs have recently been applied to the diagnosis of active tuberculosis pulmonary infection (183). Application of recombinant DNA methods to *M. tuberculosis* is in its infancy, but several investigators are planning projects. In a handful of laboratories including the National Institutes of Health and several academic institutions, MAbs are being reacted with *M. tuberculosis* to isolate and purify antigens that may be useful as diagnostic targets and may be the basis for a new tuberculosis vaccine.

Attempts are being made to isolate specific antigens for skin tests in an attempt to make tuberculin skin tests less cross-reactive to infections with other types of mycobacteria. Several tuberculosis researchers also work with *M. leprae*, the related bacterium that causes leprosy.

Research Needs

Tuberculosis may be the most serious infectious disease problem in many developing countries, as much a social as a scientific dilemma. There is an obvious need for better vaccination and diagnosis. Diagnostic methods are needed to differentiate the "atypical mycobacteria," such as *M. intracellulare* and *M. smegmatis*, which hinder diagnosis and seem to interfere with the effectiveness of BCG (Bacillus Calmette-Guerin) immunizations.

Diarrheal and Enteric Diseases

Diarrheal and enteric diseases are caused by a variety of viruses, bacteria, protozoa, and worms, and a single individual often is infected with several at one time. Full understanding of the various etiologic agents will come only when simpler techniques of diagnosis are available. Clinical diagnosis of diarrheal disease calls for immediate institution of therapy. Fortunately, rehydration therapy is appropriate for all diarrheal disease (see ch. 9). Nonetheless, identification of specific pathogens is essential for understanding the distribution of different agents and to establish priorities for specific actions.

Viral Infections

Viruses are now recognized as important agents of gastroenteritis. The principal agents are rotaviruses and Norwalk agents, though adenoviruses, astroviruses are enteroviruses, coronaviruses, and calciviruses are also found in fecal specimens (15).

Conventional Diagnosis.—Most of the viruses that cause diarrheal and enteric diseases do not grow under ordinary cell culture conditions, so direct diagnosis of viral antigen in stool specimens or detection of a serologic response is necessary. Electron microscopy is a sensitive and simple method for detecting the presence of virus (if an electron microscope is available), but only limited numbers of samples can be processed.

A variety of immunologic methods have been developed for detection of viral antigen in stool samples. The ELISA, IFA test, RIA, and CIE produce good results, though ELISA and RIA have the greatest sensitivity.

Recent Progress.—Progress has been achieved in isolating and cloning rotavirus DNA and then developing DNA hybridization assays (117). Cloned DNA hybridization probes have been used on stool specimens for rotavirus diagnosis. The utility of DNA hybridization probes in the field in developing countries must be further evaluated, but good results have been reported in detection of rotavirus in stool samples taken in remote areas of Venezuela (146).

A test "kit" for rotavirus based on an ELISA has been developed and evaluated and is now in use by more than 50 investigators in the field. A second generation ELISA test based on the use of MAbs is being developed (427).

Bacterial Infections

Conventional Diagnosis.—Definitive identification of bacteria causing diarrhea is by the isolation of the agent through culture of clinical samples (e.g., stool samples for *Shigella* and *Vibrio cholerae*; blood or stool samples for *Salmonella*. Culture of *V. cholerae* is relatively simple and gives a result in 18 hours.

Serologic diagnosis of *Salmonella typhi*, the cause of typhoid fever, is possible because specific agglutinins appear in the blood at 7 to 10 days of illness (the Widal reaction). For cholera, rise in titer of specific agglutinins or antibodies confirms the diagnosis.

Two tests for the isolation and identification of enterotoxigenic *E. coli* have been available for a number of years: one uses a miniculture of adrenal cells (302), and the other uses suckling mice (90).

Recent Progress.—Although *E. coli* is one of the most intensely studied of all organisms, with many thousands of research publications on all aspects of its biology and biochemistry, much remains to be learned about its relation to diarrheal disease. The several strains of *E. coli* that produce diarrheal and intestinal disease in humans are recognized and described on the basis of clinical pathology ("enterotoxigenic," "enteropathogenic," "enteroinvasive," see ch. 4). All of these characteristics are under genetic control, and investigators in several laboratories are identifying and cloning the genes that code for attachment and colonization, virulence, toxin production, and antibiotic resistance. With the genes in hand, diagnostic procedures can be developed.

DNA hybridization probes for field identification and typing of *E. coli* are under development in several laboratories. A DNA hybridization test for identification of enterotoxigenic *E. coli* has been available for several years (41,42) and now has been tested in the field (103,312). The DNA hybridization test has been shown to be very specific, reliable, stable, and sensitive (1,000 times more sensitive than the standard assays). It appears to be a valuable tool for epidemiologic studies.

The WHO Control of Diarrheal Diseases (CDD) program has evaluated the "Biken" gel diffusion test for detection of certain strains of *E. coli*. The test is simple to perform, accurate, reproducible, and has potential for use in developing countries. Commercial production and marketing of the materials and reagents used in the test is being pursued (427).

The CDD program is also evaluating ELISAs for diagnosis of strains of *E. coli*. Results indicate a potential for widespread application, though further development is needed to make it suitable for routine diagnostic use (427).

A DNA-DNA hybridization probe was used by one team of researchers to detect *Salmonella* bacteria not in stool samples but in food products (116). This type of application may provide far more serotype-specific identification of contaminating organisms than conventional culture methods, without the need for incubators, sterile media, and glassware.

Cholera is being studied by four or five researchers in the United States. Recombinant DNA libraries are being constructed to collect gene sequences from wild-type organisms. Studies are under way on the transmissible genetic elements isolated from *V. cholerae* from endemic areas such as Bangladesh, and differences between toxigenic and nontoxigenic organisms are being defined (16).

Campylobacter jejuni is now recognized as an important diarrheal agent, but epidemiologic study is hampered by lack of a serotyping technique. Antigenic studies are under way to develop a serotyping system. A simple slide agglutination technique to identify antigens is under evaluation in a number of developing countries (49).

Acute Respiratory Infections (ARIs)

ARIs are caused by a range of etiologic agents—viruses, bacteria, rickettsia, and parasites—presenting great diagnostic complexity. Although most of these agents can be specifically diagnosed, the process normally requires multiple serologic tests of various kinds.

Many diagnostic procedures based on isolation and culture of the organism or based on comparison of an initial and a later serum sample ("paired sera") do not provide timely enough information to be relevant to individual patient care. Also, to a large degree, the diagnosis of ARIs can be and is made on the basis of clinical symptoms, because no matter what the diagnosis, specific therapeutics are lacking, especially for the many viral diseases.

Because much of the treatment of ARIs consists of providing symptomatic relief, the identification of specific disease-causing agents is not so important. In lieu of specific diagnosis flowcharts or decision trees, using a patient's symptoms as criteria may be more appropriate for use by medical staff at all levels of the health systems of developing countries.

Still, in many cases, the etiologic agent needs to be identified. For the treatment of the individual patient, diagnosis permits rational use of available therapy. The nonspecific prophylactic or placebo use of antibiotics for any undiagnosed ARI is greatly abused. Bacterial infections can be treated with common antibiotics, but for viral agents, only symptomatic support and relief are proper (with the exception of viral influenza for which an antiviral drug is available).

For public health needs, specific diagnosis is necessary to assess, plan, implement, and evaluate interventions against epidemic outbreaks and endemic infections.

Conventional Diagnosis

Faced with an individual patient with respiratory infection, the clinician or epidemiologist must make simplifying judgments to decide which diagnostic procedures are in order. Simple microscope examination of nose or throat secretions can be useful, though limited, in ruling out possible etiologic agents.

Culture and isolation of the pathogen leads to a definitive diagnosis, but the growth takes time (usually at least 2 days), delaying the diagnosis. For viral agents and even bacterial agents, this procedure requires careful collection; special handling; storage and transport; tissue culture facilities (including appropriate culture media, and cell types for virus growth); and equipment for serologic diagnosis (e.g., immunofluorescent microscope, scintillation counter, electrophoretic equipment). Prior antibiotic treatment or contamination of the sample at any of several points from collection to inoculation, in the mature medium can easily lead to incorrect results.

Serologic diagnosis by most of the standard tests can demonstrate specific antibody in the individual's serum, but this is not definitive, since antibody from previous infections can persist after cure. Active infection is demonstrated when the serum titer of antibody in a later convalescent sample (after 1 to 3 weeks) is higher than an early acute sample ("paired sera"). This means the diagnosis is often confirmed after the infection has been resolved. This is acceptable for epidemiologic use, but not so useful for individual patient care.

Recent Progress

Viral Infections.—With the IFA test and ELISA, the diagnosis of respiratory viruses can now be made in a few hours after specimen collection (52,123,125,418). This is a great improvement over isolation by tissue culture which takes several days. With appropriate antisera, the following viral antigens can be identified by the IFA test: influenza (types A and B), respiratory syncytial virus (RSV), measles, adenovirus, parainfluenza 1,2,3,4 (125,264). The economy of time permits large numbers of specimens to be processed. ELISA appears to be very useful for detecting several viruses, but additional experience is needed to evaluate it (264).

Even with a rapid serologic diagnosis, the evidence is only indicative because of possible contamination (i.e., if the clinical sample is negative but the culture is positive, contamination of the culture is suggested rather than a positive diagnosis).

Influenza.—Because influenza infection may be fatal, and outbreaks occur annually, with major epidemics and pandemics occurring sporadically, the three main types of influenza, with numerous subtypes and strains, are monitored epidemiologically. Precise strain typing is carried out in order to prepare effective vaccines. Virus isolation and identification from tissue culture is available in 48 hours, but the definitive result may take a week or more. HI and CF tests produce a result in 24 hours, but tests of acute and convalescent serum (samples "paired sera") separated by an interval of 2 to 3 weeks provide definitive diagnosis. The neutralization test is useful but expensive and time-consuming. The direct or indirect immunofluorescent technique can be used with cells obtained from the respiratory tract for a rapid diagnosis even when the patient has no symptoms.

Respiratory Syncytial Virus (RSV).—Precise diagnosis of RSV requires culture and isolation of the virus from tissue culture, then identification with an IFA test. CF or neutralization tests can be used for serologic diagnosis in the patient. Completing the positive identification may take up to 2 weeks, though preliminary results can be obtained by early examination of the cell culture.

Fluorescent antibody tests have been developed to permit rapid diagnosis of RSV infections. If the blood sample is transported promptly to the laboratory and processed immediately, the diagnosis can be made in 4 to 6 hours (264).

Parainfluenza.—Precise diagnosis of parainfluenza viruses requires culture and isolation. Hemagglutination tests identify the virus after 5 to 10 days, while immunofluorescence tests can make the diagnosis in 24 to 72 hours. Detection of a rise in serum antibodies in the individual can be made with the HI test or CF titration, but nonspecific responses make serology an unreliable diagnostic tool. The IFA test has been used for rapid diagnosis, but it has not achieved widespread use.

Adenoviruses.—Definitive diagnosis is by culture, isolation, and use of CF, IHA, or neutralization tests. These three tests can be used to establish the diagnosis using paired serum samples from the patient.

Rhinoviruses.—For the most common agent of the common cold, with over 100 serotypes, routine serologic diagnosis is not very practical, and is not routinely available even in developed countries, though the neutralization test can be used. Even tissue culture isolation is often unsuccessful in detecting the infection, because the conditions for successful isolation require special handling (246).

Coronaviruses.—Diagnosis of coronavirus by culture isolation is not a routine procedure and needs specific types of culture cells. Serologic diagnosis can be made using the CF test.

Bacterial and Mycoplasmal Infections.—The diagnosis of bacterial agents of respiratory infections has been greatly improved over the last decade. Rapid and accurate diagnosis is now available in hours rather than days through the use of a variety of diagnostic methods to detect intact bacteria, or in some cases soluble antigen, by the COA and LA tests, ELISA, RIA, CIE, and the IFA test (264).

The COA test can be used for detection of bacteria in specimens such as sputum, serum, urine, and cerebrospinal fluid. It can also be used for strain typing of culture isolates. The test is simple, rapid, sensitive, and specific when the antiserum used is of good quality. Each bacterium to be identified needs a specific antiserum reagent with appropriate antibodies.

The LA test has been used to detect *Streptococcus pneumoniae* and *Hemophilus influenzae* in body fluids such as serum, cerebrospinal fluid, and urine. False positives result from nonspecific autoagglutinations and from reaction to antigens common to pathogenic and nonpathogenic organisms.

ELISA can be used to detect bacteria in body fluids. It is highly sensitive for detecting *H. influenzae* type B, as well as pneumococcal antigen. There are two variations: the direct assay uses a specific antibody (against the bacterial antigen) that is labeled with the enzyme. The indirect assay uses unlabeled specific antibody. The antigen-antibody complex is then identified with labeled antibody that binds to it. The indirect method is very sensitive and more useful, because it limits the need to just one labeled antibody.

RIA is extremely sensitive for detecting *S. pneumoniae* and *H. influenzae*. To evaluate the practical use of RIA, however, more experience is needed. CIE has been successfully used to detect pneumococci, streptococci, and *H. influenzae* in respiratory secretions and body fluids.

The IFA test can be used to identify bacteria but not soluble antigens. It is very sensitive for detecting *H. influenzae*, *S. pneumoniae*, and *Bordetella pertussis*. The IFA test can also be used to identify various organisms in culture.

Streptococcus pneumoniae.—Direct microscopic examination of sputum can indicate pneumococci, but the predictive value of this test is variable, because several organisms resemble pneumococci, healthy individuals can carry pneumococci, and a sputum negative for pneumococci does not rule out infection. Sputum culture is the standard procedure. Serologic diagnosis is not practical, because antibodies persist for long periods of time.

Efforts to detect antigen in respiratory tract secretions, blood, and urine by CIE (97,345), as well as by the COA and LA tests (342), have been successful.

Streptococcus pyogenes.—Diagnosis is made by isolation of the streptococci from culture of throat samples. Group determination is made with specific antiserum—the Lancefield precipitation test is considered the standard, though direct fluorescent antibody test is also useful. IFA of throat swab isolates can be obtained after 2 to 24 hours of culture. CIE can be used 6 hours after culture. The COA and LA tests have also been used. There are three convenient tests for detection of serum antibodies.

Bordetella pertussis.—Definitive diagnosis is made 2 to 3 days after culturing a throat sample on specific media, by agglutination with specific antiserum or the IFA test. A rapid diagnosis is available with the direct fluorescent antibody test.

Hemophilus influenzae.—After isolation and culture of a clinical sample, diagnosis is made by microscopically detecting a surface change in the bacterium (the Quellung test). Rapid diagnosis of antigen in secretions and body fluids is available by CIE, the LA test, and ELISA (264).

Mycoplasma pneumoniae.—Definitive diagnosis is made by culturing a throat sample on appropriate media, with subcultures made weekly for 8 weeks. When colonies appear, they can be identified visually, though IFA staining confirms the diagnosis.

Rapid diagnosis of sputum by CIE has been demonstrated, but more experience is needed to validate this technique (404). Serologic diagnosis of paired sera is done with the CF test. Detection of antibody in one sample is useless for diagnosis, because high titers persist long after initial infection.

Research Needs

There are many procedures for the diagnosis of ARIs, but various constraints reduce their practical use. Direct demonstration of causative agents is only now becoming an option. Serologic diagnosis using paired sera and serologic diagnosis of culture isolates do not provide timely information for the treatment of the individual patient. Requirements for well-equipped and well-supplied laboratory facilities are another constraint that limit the use of diagnostic procedures in tropical countries.

Continued development of the available and promising technologies for the diagnosis of ARIs is needed. With rapid, simple, reliable diagnostic tests, both the needs of the patient and the community could be better met. Ideally, such tests would not require expensive equipment, reagents, or highly trained operators. In some cases, however, if the tests have certain sophisticated requirements, it may be possible to use them in centralized laboratories.

Arboviral and Related Viral Infections

Because of the general lack of effective treatment for arboviral infections, diagnosis is of less importance to individual patients than to the community, where it is critical for recognizing outbreaks and initiating vector control measures.

Conventional Diagnosis

Clinical diagnosis of the diseases produced by the arboviruses recognizes three syndromes: 1) fevers of an undifferentiated type, frequently called "dengue-like," with or without rash and usually relatively benign; 2) encephalitis, often with a high case fatality rate; and 3) hemorrhagic fevers, also frequently severe and often fatal (418).

Conventional diagnosis of arboviral disease involves isolation of the virus in newborn mice or cell culture, followed by serologic diagnosis using HI, CF, or neutralization tests. The serologic diagnosis itself is quick and straightforward, but successful culture requires appropriate laboratory equipment, tissue culture materials, and time (usually at least 2 days). More sensitive cell cultures have become available using mosquito cell lines from which early detection can be made using the IFA test (340). It is also possible to inoculate clinical samples into live mosquitoes and then identify the virus using an IFA test of the salivary glands. This technique is very sensitive, but slow (10 to 14 days) (190). Other new methods, such as the RPHA test, are being developed to detect the virus earlier in the culture cycle.

Serologic diagnosis with paired sera still uses conventional methods (HI, CF, and neutralization tests). Single radial hemolysis has been introduced with some important advantages (124). It is as sensitive as the HI test for dengue fever, tick-borne encephalitis, yellow fever, West Nile fever, and Venezuelan equine encephalitis, yet simpler to perform. The method looks promising but needs further evaluation with different viruses.

Direct detection of virus from the patient is dependent on sufficient virus in specimens. The IFA test has been used with some success for a few diseases (Japanese B encephalitis, Colorado tick fever, Rift Valley fever). ELISA and RPHA methods are being evaluated for detection of viral antigen in body fluids and respiratory secretions. CIE has been used to detect dengue virus in sera from patients with acute diseases but the test has low sensitivity. In all cases of individual direct diagnosis, a negative result is not conclusive.

Detection of specific IgM antibody (which is an early immune response in the acute phase of infection) is carried out by the HI test, ELISA, and the IFA test. This is used for the diagnosis in convalescent patients (when virus has disappeared) and for primary dengue virus.

Recent Progress

MAbs have been developed for certain of the Togaviruses, Bunyaviruses, and Arenaviruses, and nucleic acid hybridization techniques are in various stages of development for some of these viruses. ELISAs are also being developed for members of each group and field tests for some have begun. MAbs to antigens common to groups of viruses have been developed which allow "generic" diagnosis of disease, frequently sufficient to initiate medical therapy and epidemic prevention measures (197).

MAbs for early type-specific identification of the four main serotypes of dengue viruses have been developed by the U.S. Army at the Walter Reed Army Institute of Research (197) and are now generally available. However, dengue virus isolation is still difficult as most patients have low virus concentrations, and the viruses grow poorly in cell cultures. The U.S. Public Health Service laboratory in Fort Collins, CO, also is producing dengue monoclonals in collaboration with WHO. MAbs have also been made to specific surface antigens of several viruses, and ELISA tests based on these reagents are being evaluated (16).

Research Needs

Development of synthetic peptides following sequence analyses of the alphaviruses and flaviviruses could be very important for developing diagnostic reagents to detect serum antibodies (as well as for vaccine development). With regard to the needs of individual patients, rapid diagnostic methods will become increasingly important as drug treatments for arboviruses are developed.

SUMMARY

There is great variability in the availability of diagnostic technologies for diseases of importance in developing countries. In general, however, lack of effective diagnosis is a major obstacle to health care only when health care systems are adequate to act on diagnoses. This situation is not the norm today. While diagnosis is an integral part of medical care, in some cases, diagnostic technologies have even greater value in providing information about the incidence, prevalence, and natural history of diseases of importance to developing countries. Development and use of diagnostic technologies in research could lead to effective integrated disease control strategies.

9.

Therapeutic Technologies: Selected Tropical Diseases

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9.

Therapeutic Technologies: Selected Tropical Diseases

INTRODUCTION

The purpose of therapy is to alter the course of disease so that its consequences are less severe, or to make being ill more tolerable for the patient. Therapy for infections may treat symptoms, which themselves can be dangerous, or may directly attack the responsible organism.

Symptomatic treatment is quite common. Thus, for instance, it is usual to take measures against high fevers, regardless of the cause, because very high body temperatures can result in brain damage. There may or may not be treatment to eliminate the organism causing the infection, and the organism may not even be identified; nevertheless, controlling fever is important. Another symptomatic treatment that has quickly become one of the most potent tools in the tropics is oral rehydration therapy (ORT) for the dehydration that accompanies diarrheal diseases (see *Case Study A: Oral Rehydration Therapy for Diarrheal Diseases*).

Symptomatic treatment is adequate for some infections that are self-limited. For infections that the body cannot eliminate, however, the goal of therapy is the eradication of the disease-producing organisms, not simply alleviation of symptoms, although this goal is difficult if not impossible to achieve for many tropical diseases.

In general, therapy against bacterial infections is safe, effective, and usually lasts about 1 week. Some bacterial infections (e.g., urinary tract infections) can be treated with one dose; others (e.g., those causing enlargement of the heart, endocarditis) may require 6 weeks of therapy. Prolonged treatment, usually 6 months or more, is the norm for tuberculosis, and lifetime treatment is necessary in the case of lepromatous leprosy. Many tropical diseases caused by helminths can now be adequately treated with 1 to 6 days of therapy, while for others, there is no adequate therapy. The treatment for diseases caused by protozoa is similar. The development of antiviral drugs is still in its infancy.

The mere existence of adequate therapy does not guarantee that it will be used. The availability and quality of health care varies from country to country and from one region of a country to another. Some areas in developing countries, principally the cities, have quite modern health facilities; other areas have only dispensaries or often nothing at all. This variability results in a marked inconsistency in the ability to make specific diagnoses and administer pathogen-directed treatments. In most developing countries, antimicrobials are readily available over-the-counter. The result is two common abuses of these drugs: use for conditions in which they are ineffective and use in inadequate doses. Both extensive use and underdosing promote drug resistance in the pathogens. The prevalence of organisms resistant to the antimicrobials most commonly available is high and thus poses significant therapeutic problems.

The antimicrobials that are generally available in developing countries are penicillin, chloramphenicol, various sulfonamides, tetracycline, streptomycin, isoniazid (INH), chloroquine, pyrimethamine-sulfadoxine (P/S), and some antihelminthic drugs. These are relatively inexpensive compared to other, newer agents, but have significant drawbacks. It is to these agents that resistance in some areas is widespread and growing. Alternatives are usually marketed, but frequently not available where they are needed, often because they are too expensive. Many of the older drugs, particularly the antihelminthics, are toxic to the patient. Drugs for chronic infections often require months or years of treatment, so their effective use in developing countries, particularly in rural areas, is unlikely.

Some progress is being made. Older chemotherapeutic agents are being reexamined; newer ones are being screened in the laboratory and in animals. The process of drug development is slow, however, and a decade can easily elapse before a promising chemical is marketed as an approved drug. There is a great need for safe, effective, inexpensive, oral, single dose therapies for tropical diseases. Alternatives to the present toxic agents and alternatives for use against drug-resistant organisms are particularly pressing needs. A major problem with new drug development for important pathogens in developing countries is that some diseases are relatively rare and others are prevalent only in areas with limited economical resources. Thus, financial incentives for the pharmaceutical companies best equipped to develop new agents are lacking.

THERAPIES: CURRENT STATUS FOR SELECTED TROPICAL DISEASES

Malaria

Human malaria is caused by four species of the genus *Plasmodium*: *P. falciparum*, *P. malariae*, *P. vivax*, and *P. ovale*. There are many groups of drugs available for the prevention and treatment of this disease. Their use is determined by the stage of disease, the immunologic status of the patient, and the probability that the parasite is susceptible to a particular drug (usually geographically determined). Some drugs are effective against sporozoites (the invasive stage of *Plasmodium*), some against hypnozoites (the latent stage of *P. vivax* and *P. ovale* in the liver), some against the merozoites (the erythrocytic or red blood cell stage), and still others against gametocytes (the sexual form which is picked up by the mosquito during feeding and perpetuates malaria transmission after further development in the mosquito). Some of the drugs affect more than one stage of the parasite.

Resistance of *P. falciparum* to most agents is growing. Resistance of *P. falciparum* to chloroquine has been present for some years in Panama, parts of some South American countries, India, Southeast Asia, Indonesia, China, the Republic of the Philippines, and other Pacific islands. It has spread from some parts of South America to include most parts of Bolivia, Venezuela, French Guyana, and northern Peru. There is growing prevalence of resistance in east Africa, which includes Kenya, Tanzania, eastern Zaire, Burundi, Uganda, Rwanda, Malawi, Zambia, northern Sudan, Madagascar, and the Comoro Islands (377).

In some areas, *P. falciparum* strains are also resistant to drugs other than chloroquine, such as P/S, quinine, and mefloquine (see below). Strains resistant to P/S were thought originally to be present only in Southeast Asia. Now there appear to be some resistance in Kenya, Tanzania, and the Amazon basin. Most of these are resistant to both chloroquine and P/S. Some strains in Southeast Asia are resistant to chloroquine, P/S, and mefloquine, and relatively resistant to quinine. These multiply resistant strains are mostly limited to the Thailand-Kampuchean border.

Agents for Treatment of Acute Malaria

Specific chemotherapy for acute malaria is at least 400 years old. During the 1600s, it was known that the bark of a Peruvian tree, the cinchona tree, was effective in the treatment of intermittent fever. The active ingredient, quinine, was isolated in 1820 by two French chemists, Pelletier and Caventou. Since then, new compounds have been discovered that are useful in the treatment of malaria; however, the ideal drug is yet to be discovered. The treatment of malaria becomes less satisfactory almost every year, mainly because more areas of the world report *P. falciparum* resistant to currently available drugs and drugs in clinical trials.

Quinine.—Quinine was the first medication for the treatment of malaria, and it continues to play a key role. Quinine is effective mainly against the merozoite stage of *Plasmodium* and therefore is effective in the treatment of acute malaria of all species. It was the sole specific chemotherapeutic agent for the treatment of acute malaria until World War I. As new drugs such as quinacrine (1930) and chloroquine (1934) were discovered, the importance and use of quinine diminished. With the emergence of chloroquine-resistant, quinine-sensitive strains of malaria parasites in 1959 in Venezuela (274), however, quinine has again become invaluable, and recent research has helped clarify its proper use.

Quinine is used parenterally (by injection) or orally as the drug of first choice in patients with falciparum malaria in areas of the world where chloroquine-resistant, P/S-resistant *P. falciparum* is prevalent. Unfortunately, it is the most toxic of all antimalarials used regularly to treat acute attacks, and this toxicity limits its use. Adverse effects from quinine are relatively common, ranging from sudden death (which is rare, but may occur from rapid intravenous infusion or hypersensitivity), to more common ringing in the ears, nausea, visual disturbance, and headache. Quinine is also associated with hypoglycemia (low blood sugar), hemolytic anemia (breakdown of red blood cells), and a decrease in clotting factors. Furthermore, increasing resistance of *P. falciparum* to quinine has recently been reported in Thailand and has been associated with treatment failures when quinine has been administered in standard doses (48).

4-Aminoquinolines.—Since quinine (and quinacrine) was in limited supply during World War II and was quite toxic, efforts were made to find alternatives. Through a cooperative research program in the United States during World War II, investigation of 4-aminoquinolines was undertaken after one of these compounds was reported by the French to be well tolerated and highly active.

Chloroquine.—Chloroquine is the most valuable and most extensively used of the 4-aminoquinolines. It has activity mainly against the merozoite. It continues to be the drug of first choice for treatment of acute malaria in "nonallergic" persons with chloroquine-susceptible strains of the malaria parasite and for prophylaxis for travelers in areas without chloroquine-resistant strains. Chloroquine can be given orally, intramuscularly, or intravenously and is generally available in the geographical areas where it is needed. It is well tolerated in the usual treatment and prophylactic dosages. Occasional side effects include mild transient headaches, nausea, diarrhea, visual disturbances, and pruritus (itching). Pruritis may occur in anyone, but more commonly in blacks. It probably does not cause birth defects, and is therefore presumed to be safe during pregnancy.

Amodiaquine.—Amodiaquine, another 4-aminoquinoline, was first found to be effective against nonhuman malaria in 1946. It, like chloroquine, is effective in preventing acute malaria and in treating patients with acute malaria due to all four species of *Plasmodium*, except chloroquine-resistant strains of *P. falciparum*. Amodiaquine is slightly more effective than chloroquine against these resistant strains; however, this fact probably has little clinical significance (328). The side effects of amodiaquine are similar to, but possibly slightly less severe than, those of chloroquine. Amodiaquine is available only in an oral form, and since it does not have a bitter taste like chloroquine, it is more acceptable to children.

Pyrimethamine-Sulfadoxine.—It appeared for a short period of time that the introduction of chloroquine (1934) had brought to an end the search for malaria treatment. Then resistance to chloroquine was reported. Again an extensive search began for drugs effective against chloroquine-resistant strains of *P. falciparum*. Many previously available drugs were screened for activity against malaria parasites. In course, it was discovered that by combining pyrimethamine with a sulfa derivative, pyrimethamine resistance could be overcome. Thus, P/S (Fansidar) was marketed as another effective regimen in the treatment and prevention of acute malaria due to all four species, excluding P/S-resistant *P. falciparum* strains.

P/S activity is against the merozoite. This combination was, and still is, one of the most widely used regimens for the treatment of uncomplicated chloroquine-resistant *P. falciparum* infections. P/S is also frequently used in Africa for the treatment of chloroquine-sensitive acute malaria. Blacks frequently have itching from chloroquine and therefore use P/S as a substitute. Unfortunately, others without chloroquine-induced itching continue to use P/S when chloroquine would be adequate. Many strains of chloroquine-resistant *P. falciparum* are now resistant to P/S. This situation is thought to result partially from the indiscriminate use of P/S for prophylaxis and partially from treatment with inadequate doses.

Adverse effects are those related to pyrimethamine and sulfadoxine individually. Pyrimethamine has few side effects. Sulfadoxine has the usual adverse effects associated with any sulfa, including gastrointestinal and skin reactions which may be severe, and can be life threatening in persons with sulfa allergy. P/S has not been available in all countries, and it has only recently become available in a parenteral form.

Pyrimethamine has also been combined with other sulfas (sulfalene, dapsone) both for treatment and prophylaxis.

Trimethoprim.—Sulfas have also been combined with trimethoprim for use mainly in bacterial infections, and these combinations have been used with quinine in the treatment of uncomplicated chloroquine-resistant (known or suspected) *P. falciparum*. Trimethoprim's main activity is against the red blood cell stage of *Plasmodium*. It has relatively few side effects at doses usually given for malaria.

Tetracycline.—Tetracyclines are antibacterial agents available in various forms since 1948. They do have activity against the red blood cell form of *Plasmodium*, but this effect is extremely slow. They are not used alone, but mainly serve as adjuncts to quinine in the treatment of chloroquine-resistant, P/S-resistant *P. falciparum* infections.

Alteration of the intestinal flora usually occurs within 48 hours following daily administration of the usual therapeutic dosages. This alteration of the normal intestinal flora increases one's susceptibility to enteric (gut) pathogenic bacteria such as *Salmonella*. Tetracyclines also sensitize the skin to sunburn, besides causing nausea, vomiting, diarrhea, and discoloration of the teeth during development, the latter limiting their usefulness in children and pregnant women.

Quinacrine (Mepacrine).—Quinacrine is essentially an obsolete antimalarial because of its side effects. It is important historically because of its widespread use during World War II.

Mefloquine.—Mefloquine is one of a number of 4-quinolinemethanols developed during the 1970s by the U.S. Army Research and Development Command. This group of drugs has been studied since World War II. Mefloquine, the most active of this group, has been found effective in both prophylaxis and treatment of acute malaria due to all species, including chloroquine-resistant, P/S-resistant *P. falciparum* infections. Unfortunately, there already have been reports of resistance of *P. falciparum* strains to mefloquine both in vivo and in vitro (321). Thus, when used prophylactically, mefloquine is combined with P/S.

Agents for the Treatment of Persistent Malaria (Radical Cure)

So far, this discussion has focused on the drugs used for treating acute clinical malaria. Of the four species of *Plasmodium* that cause malaria, only two, *P. vivax* and *P. ovale*, have persistent liver stages. Plasmodial forms in the liver are called hypnozoites because they can remain quiescent for long periods, up to 3 years in cases of *P. vivax*. Following subsequent development, hypnozoites can rupture out of the liver and invade red blood cells (erythrocytes), producing clinical malaria. While hypnozoites are in the liver, patients are asymptomatic.

Quinine, chloroquine, mefloquine, and most of the other antimalarials effective in treating acute malaria have no effect on the liver stages. Since susceptible *P. falciparum* and *P. malariae* do not have liver stages, these infections can be cured by standard treatment regimens effective against the merozoites. However, since *P. vivax* and *P. ovale* do have liver stages, these infections can recur following treatment with agents effective only against the erythrocytic form.

In 1924, an 8-aminoquinoline, pamaquine, was found to be effective in the treatment of malaria. Too toxic for general use, it has been supplanted by a drug in the same family, primaquine (1950). Primaquine has good activity against the liver stages of *P. vivax* and *P. ovale* and currently is the only drug besides quinocide, another of the same family, which is effective in eliminating the hypnozoites. Although both pamaquine and primaquine also have activity against the erythrocytic stages, the necessary doses are toxic. Unfortunately, some strains require increased doses and duration of therapy for complete elimination of the hypnozoites (115).

Primaquine's use is limited by gastrointestinal symptoms and hemolytic anemia. Hemolytic anemia occurs in people with a deficiency of a certain enzyme, G6PD, most frequently occurring in blacks and people of Asian or Middle Eastern descent. Hemolysis in blacks is usually self-limited; however, in Asians, hemolysis may not resolve even after primaquine is withdrawn.

Agents for Malaria Prophylaxis

Drugs that *prevent* acute malaria are said to be used as prophylaxis. Some of these, chloroquine and P/S, have already been mentioned. Chloroquine is effective in preventing multiplication of merozoites. P/S is effective against the sporozoites and merozoites. Unfortunately, again, both chloroquine and P/S are limited in geographical use because of drug resistance.

Another drug, proguanil (chloroguanide) has been available since 1945 and has been effective in the suppression and treatment of both *P. vivax* and *P. falciparum* infections. Like pyrimethamine, proguanil now has limited use because of the emergence of resistant strains of *P. falciparum*. It is not useful for the treatment of acute attacks, but may be used as prophylaxis in areas where *P. falciparum* is susceptible to it. Not infrequently, resistance to pyrimethamine and proguanil exist together.

Recent Advances in the Supportive Treatment of Malaria

Advances in the treatment of severe malaria apart from antimalarials include the discovery of the deleterious effect of steroids, used previously in the treatment of cerebral malaria (395), hypoglycemia associated with severe malaria and quinine therapy (148,228,403), quinine pharmacokinetics (absorption, distribution, and excretion of the drug) (401,402), and the effectiveness of exchange transfusion for high levels of parasitemia (191).

Summary of Current Malaria Therapy and Outlook for the Future

The armamentarium of chemotherapeutic agents to be used against malaria is *barely* adequate in areas of the world where there are resistant strains of *P. falciparum*. Strains of *P. falciparum* resistant to chloroquine and P/S are spreading (327). Quinine and mefloquine resistance is emerging.

In areas where there are both chloroquine- and P/S-resistant strains of malaria parasites, no effective prophylaxis is available except for quinine and mefloquine. As these drugs are used for prophylaxis, and used in inadequate doses, as so frequently happens in developing countries, resistance to them will probably increase. Primaquine is an effective cure for the hypnozoite stage; however, the increased dosage required for some strains suggest that its effectiveness may be diminishing. (Strains such as the "Chesson strain" are thought to have *intrinsic* resistance, in contrast to resistance acquired from drug exposure.) It is clear that new effective antimalarial agents are needed as alternatives for most of the ones currently available.

Schistosomiasis

Recently, the treatment for all forms of schistosomiasis has changed significantly. Antimony potassium tartrate was initially found to be effective in 1918. It was subsequently replaced by trivalent antimonials such as stibophen, and these were largely replaced by hycanthone (1960s) and niridazole (1966), which were the drugs of choice until recently. Three agents which have been studied since about the 1970s—metrifonate, oxamniquine, and praziquantel—are effective given orally and have few adverse effects compared to hycanthone and niridazole, but are expensive. Were it not for the expense, these agents would probably replace hycanthone and niridazole in the treatment of schistosomiasis.

Hycanthone and Niridazole.--Hycanthone is effective against *Schistosoma mansoni* and *S. haematobium*, but must be given intramuscularly in a single dose and commonly causes nausea and diarrhea. Niridazole can be given orally, is effective against *S. mansoni, S. haematobium* and somewhat against *S. japonicum*, but may cause neurologic side effects necessitating observation during therapy.

Metrifonate.—Metrifonate is effective only against *S. haematobium*. It can be administered orally, but must be given three times separated by 2-week intervals and its effectiveness varies greatly.

Oxamniquine.—Oxamniquine, effective against *S. mansoni*, may be given orally. Side effects are usually mild. Strains of *S. mansoni* vary in susceptibility, from highly susceptible strains in the Western Hemisphere, requiring only one dose, to less susceptible strains in Africa, requiring about four times the dose given over a 2- to 3-day period.

Praziquantel.—Praziquantel is a welcome addition to the antischistosomal armamentarium. Effective against all known human schistosomes and many other trematodes, it can also be given in one oral dose and is well tolerated. Cure rates range from 70 to 95 percent with *S. mansoni* and *S. haematobium*.

Oltipraz.—Oltipraz, now undergoing clinical trials, appears to have promise for the future. It is well tolerated and has produced cure rates of greater than 80 percent with both *S. hematobium* and *S. mansoni*.

Amosconate.—Amosconate, a new antischistosomal drug, has demonstrated efficacy against *S. mansoni* and *S. japonicum* and is effective against *S. haematobium* infections of primates besides other worms. It also has some serious side effects.

Trypanosomiasis

African Sleeping Sickness (African Trypanosomiasis)

Therapy for African sleeping sickness, whether due to *Trypanosoma brucei gambiense* or to *T.b. rhodesiense*, differs depending on the stage of the disease.

The early stage of African sleeping sickness can be treated with suramin, which has been available since the 1920s. It is given on days 1, 3, 7, 14, and 21 slowly intravenously, and then weekly twice after that. Suramin has serious side effects, the immediate ones consisting of nausea, vomiting, seizures, loss of consciousness, and shock. The most important delayed effect is kidney damage. Other side effects have been reported. The drug is not effective once the pathogenic organisms have invaded the central nervous system.

Another drug that is effective in the early stage is pentamidine, which has been available since the mid-1930s. Pentamidine's use is limited by side effects which frequently result in an abbreviated course of therapy. The drug must be administered intramuscularly, resulting in pain at the injection sites. It can also cause abscesses, hypoglycemia (low blood sugar) or hyperglycemia (high blood sugar), pancreatitis, and hypotension (low blood pressure) if inadvertently given intravenously. Pentamidine is generally not as effective as suramin.

The late stage of African trypanosomiasis can be treated with melarsoprol, an arsenical first shown to have activity against trypanosomes in 1940. Melarsoprol is effective against both subspecies and both stages of disease, but its use is limited by its toxicity. This agent is used only after therapy with other agents has failed. It must be given intravenously daily, or every other day, for three doses, and then this sequence must be repeated twice more after some time has elapsed. Side effects include intensely irritating local reactions upon leakage into tissue and a potentially fatal reaction of the brain.

In summary, therapy for African sleeping sickness requires the use of toxic and frequently only partially effective drugs over an extended period of time. Hospitalization is usually required for administration and observation of intravenous therapy. Effective and appropriate treatment will require new drugs that require short courses, and are easy to administer. Such treatment is not now foreseeable.

Chagas' Disease (American Trypanosomiasis)

Chagas' disease has two clinical stages: acute and chronic. The acute stage can be suppressed by the drug nifurtimox, which has been available since the 1970s. Following treatment with nifurtimox for 120 days, one study has claimed an 80-percent cure rate at 2 years (258). However, strains of *T. cruzi* vary in susceptibility depending on the area—strains from Argentina and Chile are more susceptible than some Brazilian strains.

Nifurtimox is fairly well tolerated, although side effects such as gastrointestinal upset and abnormalities of nerves occur in 40 to 70 percent of patients. Another drug reported to be effective in the acute stage in a large percentage of patients is benzonidazole (258). Both are given orally.

Leishmaniasis

Leishmaniasis, caused by protozoans of the genus *Leishmania*, has three major forms, depending on the species of parasite: 1) cutaneous leishmaniasis, a localized ulcer caused by one of three species, *L. tropica*, *L. mexicani*, or *L. braziliensis* (determined by the geographical location); 2) mucocutaneous leishmaniasis, an invasive destructive lesion caused by *L. braziliensis*; and 3) visceral leishmaniasis, a systemic disease caused by *L. donovani*. Visceral, mucocutaneous, and complicated cutaneous leishmaniasis require therapy.

Antimonials.—Antimony-containing compounds have been the mainstays of therapy for all types of leishmaniasis requiring treatment since tartar emetic was shown to be effective in 1912 (114). Sodium stibogluconate was found effective as early as 1937 and continues to be the treatment of choice along with meglumine antimonate. Both of these drugs must be given parenterally for 10 to 30 days depending on the susceptibility of the *Leishmania* parasites.

Appropriate regimens of therapy for each strain from each geographic area have not been precisely determined. Ten-day periods of treatment are effective in the majority of patients in China, India, and the Sudan, but persons infected with Kenyan strains usually receive a 30-day course. Therapy is frequently interrupted by a rest period of 10 to 14 days.

Recently, therapy in India has been increased from 10 to 20 days, decreasing the relapse rate from 13 to 0.5 percent. Also, the pharmacokinetics of sodium stibogluconate have recently been studied, suggesting that daily doses need to be increased.

Side effects of nausea, anorexia, and malaise are generally tolerable. Antimonials do cause changes in the electrocardiogram, generally in doses higher than those used against leishmaniasis.

Amphotericin.—Amphotericin B1DD, an agent used in the United States mainly for its antifungal activity, has also been found to be effective against leishmaniasis. It must be given intravenously, usually every other day or daily, until 2.5 grams total dose is achieved. This dose takes at least 1 month to administer. Because amphotericin is extremely toxic to the kidneys, renal function must be monitored. The facilities to do this are frequently not available where the patients are in developing countries.

Pentamidine and Stilbamidine.—Another agent useful in leishmaniasis is pentamidine. As mentioned previously in relation to trypanosomiasis, its use is limited by its side effects. A similar compound, stilbamidine, may be more effective, but its use is limited by its effects on the nervous system. Hydroxystilbamidine is also effective, but not generally available in developing countries.

Allopurinol Riboside.—Allopurinol riboside is a drug most often used in the United States to decrease uric acid excretion by the kidney in the treatment of gout. A derivative has shown some in vitro activity against *L. donovani* and is almost ready to undergo clinical trials in cutaneous leishmaniasis at the Gorgas Memorial Laboratory.

Other Agents.—The role, if any, of other agents such as cycloguanil, pyrimethamine, metronidazole, benzimidazole, rifampin, and nitrofurtimox remains to be clarified.

Summary

At present, management of leishmaniasis is usually hospital-based, since most therapy is parenteral, and side effects common and frequently serious. There is, therefore, a need for effective, safe, oral agents in the treatment of these diseases.

Filariasis

There are at least eight types of human filarial infections. The three most important are infections by *Wuchereria bancrofti*, *Brugia malayi*, and *Onchocerca volvulus*. The therapy for all filarial infections is similar. There is effective therapy against microfilariae (the immature worms that cause the symptoms of filariasis), but no nontoxic therapy is available against all the species of adult worms.

Diethylcarbamazine.—Diethylcarbamazine kills microfilariae and is therefore effective as prophylaxis or in treatment of symptomatic infections. It also has an effect on the adults of *W. bancrofti* and *Loa loa* (the agent of African eyeworm disease). Diethylcarbamazine can be given orally. Side effects include adverse diethylcarbamazine reactions to itself and reactions to dying worms. The latter can be severe and exacerbate eye damage.

Suramin.—Suramin is also effective against microfilariae and adults of *O. volvulus*. Following an initial test dose, there must be 1 week of observation. If no adverse reactions occur, doses may be increased at weekly intervals. Therapy usually requires 6 weeks. Suramin must be given intravenously. Side effects include adverse reactions to the drug itself and reactions to the dying worms.

New Drugs.—New drugs undergoing evaluation are ivermectin, mebendazole, and flubendazole. The early results with ivermectin are very encouraging. A report of the first study of ivermectin in human beings with onchocerciasis (river blindness) was published in 1982 (14). The side effects that occur with the existing drugs were absent, and ivermectin cleared or greatly reduced the microfilarial load in all 32 subjects after one dose. Those results have been duplicated in at least one subsequent study (14,77). Both mebendazole and flubendazole have activity against adult worms.

Summary

In summary, there is a great need for inexpensive, well-tolerated, oral agents effective against adult worms. Of the new drugs under consideration, ivermectin may ultimately have the potential for controlling filariasis in endemic areas, but that prospect is not near at hand.

Leprosy (Hansen's Disease)

Treatment for leprosy varies depending on the severity of disease, which ranges from tuberculoid leprosy (localized lesions with few *Mycobacterium leprae* organisms) to lepromatous leprosy (which is widespread disease and infection with large numbers of *M. leprae* bacteria). The course of treatment ranges from about 3 years for some patients at the tuberculoid end of the spectrum who have relatively good immunity to lifetime treatment for patients with lepromatous leprosy who have little or no immunity.

Dapsone.—Specific chemotherapy for leprosy was not available until 1940, when dapsone, a sulfa derivative, was shown to be effective when administered by injection to patients at the U.S. Public Health Service leprosarium at Carville, LA. Dapsone was shown to be effective when given orally in 1947 and continues to be an effective, safe, inexpensive agent. Acedapsone is an injected drug which releases dapsone slowly. Both have the usual side effects of any sulfa-containing drug.

Dapsone is bacteriostatic, able to inhibit, but not kill, *M. leprae*. Viable dapsone-susceptible bacilli can be isolated from patients after 10 years of treatment even though they have no evidence of active disease.

Resistance to dapsone, first reported in 1964, is now an important problem. Up to 62.5 percent of newly diagnosed and 9.3 percent of previously treated patients have resistant strains of *M. leprae*.

Resistance to dapsone develops, like resistance to many antimicrobials, when patients do not follow the prescribed treatment regimens, for whatever reasons. Irregular compliance with treatment frequently occurs as the result of unsupervised administration. It is hoped that combination chemotherapy (see below) will minimize the development of resistance. Development of widespread resistance could lead to loss of control of leprosy in a community.

Clofazimine.—Clofazimine is another bacteriostatic agent with a unique additional property of being anti-inflammatory. Its use is limited by its cost and side effects, consisting mainly of red skin pigmentation, nausea, and diarrhea.

Rifampin.—The only bactericidal (able to kill bacteria) agent available for the treatment of leprosy is rifampin. It is rapidly effective in reducing the number of bacteria. However, even after 5 years of treatment, viable bacteria can be recovered from patients. Its use again is limited by its cost and side effects.

Combination Chemotherapy.—Since viable bacteria can be recovered from patients treated with all of the agents mentioned above, and since dapsone resistance is quite prevalent, combinations of the above drugs are now recommended as standard treatment.

Summary

The difficulties in treating people with leprosy are readily apparent. Treatment with the agents that are currently available necessitates many years of supervised chemotherapy. Cure is virtually impossible in many patients who require lifetime treatment. When dapsone resistance develops, there are few effective alternatives. There is great need for new bactericidal agents. Since recovery from leprosy will probably not occur until the body's natural defenses against the organism are adequate, immunopotentiating agents, drugs able to stimulate the natural defenses of the body, may be the solution in the future.

Tuberculosis

It has been 40 years since the discovery that streptomycin was effective in arresting the growth of *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis. Two years later, para-aminosalicylic acid (PAS) was also found to be an effective "tuberculostatic" agent, arresting the growth of the bacteria, but not killing them. With the development of *M. tuberculosis* bacteria resistant to streptomycin in 1947, PAS became the companion drug of streptomycin to prevent the growth of resistant organisms. This combination was only partially effective in eliminating resistant strains.

It was not until 1952, with the introduction of the tuberculocidal drug INH (isoniazid) that the era of effective chemotherapy for tuberculosis began. INH actually kills the organisms, rather than holding them at bay. INH is not adequate as monotherapy, however, because of the development of resistant organisms. For that reason, INH was combined with PAS or streptomycin. In 1968, with the introduction of ethambutol, another tuberculostatic agent, antituberculosis therapy seemed adequate and safe. Because the duration of therapy was long, 12 to 24 months, however, patient compliance with the treatment regimen remained a problem.

Rifampin was introduced in 1971, but its special advantage, rapid tuberculocidal activity, was not realized until the late 1970s. Rifampin helps cure the patient, and it reduces the period of time during which the patient can transmit disease to others. *M. tuberculosis* bacteria are transmitted in sputum, particularly when the patient coughs. Combining rifampin with INH rapidly clears the sputum of bacteria, decreasing the infectivity of the patient and thereby decreasing transmission of disease. The duration of therapy has been reduced from the previously recommended 18 months with INH and ethambutol to 9 months with INH and rifampin. INH is prohibitively expensive for use in many less developed countries, however, and for that reason, simply is not available where it is most needed.

Short-Course Chemotherapy.—Abbreviated courses of therapy for tuberculosis are an important goal because such courses increase patient compliance with the treatment regimen. Inadequate treatment promotes the growth of drug-resistant organisms, and failure to achieve cure promotes spread of the disease.

In an effort to reduce the duration of therapy, various regimens of short-course combination chemotherapy have been tested in developing countries, many sponsored by the British Medical Research Council. These courses primarily involve combinations of INH, rifampin, pyrazinamide, and streptomycin. The results indicate that duration of therapy may be reduced to 6 months or possibly even less when these drugs are used in certain combinations.

Although compliance problems are less with shorter courses of therapy, they have not been eliminated. Liver toxicity continues to occur with most drug combinations, but usually does not require discontinuing of therapy.

Problem of Resistance.—It is fortunate that agents described above are so effective in treating most patients with tuberculosis. For tuberculosis patients with resistant organisms, however, therapy is only barely adequate. For these patients, more drugs are required, resulting in more side effects, problems of compliance, and higher cost. Isolation of INH-resistant organisms from newly diagnosed cases of tuberculosis is common in the developing world, and there are some bacilli resistant to both INH and streptomycin (101).

The main limitation in these areas, however, is not drug resistance but cost. Rifampin is very expensive for most developing countries with high prevalences of tuberculosis. Rifampin treatment for an adult costs $0.44 to $1.60/day, INH $0.001 to $0.004/day, pyrazinamide $0.09 to $0.72/day (53). In a 9-month course (2 months of ethambutol, INH, and rifampin followed by 7 months of INH and rifampin), rifampin accounted for 96 percent of the cost of the regimen (121). If this regimen were used, for example, in the Philippines in 1976, it would have cost about $200/patient (121). With an estimated 141,040 persons needing therapy, the total cost of treatment would be approximately $28 million. These countries, therefore, continue to use relatively long courses of less expensive chemotherapeutic agents, with predictable compliance problems and a predictable lack of control of tuberculosis.

Summary

In summary, there is adequate treatment available now for most tuberculosis. Although effective agents are available, many countries with tropical environments are unable to benefit from them because of financial restrictions and compliance problems.

A need for new drugs, particularly to be used in combination with INH, however, remains. The current companion drugs have significant toxicities or are associated with compliance problems. There are drug-resistant strains of *M. tuberculosis*. Because some strains are resistant to more than one agent, there is a need for new effective tuberculocidal agents with rapid onset of action, especially as an alternative to INH and rifampin.

Diarrheal and Enteric Diseases

General Treatment

Until the late 1960s, treatment of moderate diarrhea required hospitalization and intravenous hydration therapy. In 1964, it was shown that patients with cholera responded well to oral rehydration with glucose, bicarbonate, potassium, and sodium chloride (276). Three years later, it was shown that glucose facilitated the absorption of these electrolytes and decreased the total stool volume (337). In 1968, oral administration of a solution containing glucose and the electrolytes mentioned above was demonstrated to be effective therapy for moderate dehydration secondary to cholera (250). Since then, much has been written concerning the role of ORT (oral rehydration therapy). WHO now has made recommendations for the composition of the oral rehydration solution. In 1983, 29 million packs containing the ingredients to be mixed with water for ORT were supplied to various countries.

ORT has made a tremendous impact on the resources needed to manage diarrheal illnesses, particularly in infants and children (see *Case Study A: Oral Rehydration Therapy for Diarrheal Diseases*). The oral rehydration solutions can be given by mothers at home and mothers can be trained by paramedical personnel, thus saving both the expense of hospitalization and time of the medi-

Photo credit: Dr. Robert Edelman, National Institutes of Health

Intravenous rehydration for most cholera patients, shown here, is being replaced by the simpler, more cost-effective alternative, oral rehydration therapy (ORT).

cal personnel. About 95 percent of all patients with diarrhea given ORT respond. Those who do not can be treated with intravenous solutions. Severely dehydrated patients, however, require initial intravenous therapy. ORT is effective in diarrheas from many causes. The success of the therapy depends significantly on the cooperation of the patient and/or mother. In some areas, tradition still impedes behavioral changes necessary for successful ORT. In many areas, sanitary water is unavailable. Water must be boiled prior to making the oral rehydration solution, and mothers frequently may be reluctant to do this.

Treatment for Specific Organisms

Viruses.—Viruses cause a significant amount of diarrheal illnesses in the tropics. Viral agents of diarrheal diseases include rotavirus and Norwalk agent, possibly the two most important agents, adenoviruses, enteroviruses, etc., as well as other agents which have not been specifically identified.

There is no effective specific therapy for any of the viral agents of diarrheal diseases. None of the generally available antiviral agents are useful in treating diarrheal illnesses. Interferon, a molecule which can protect host cells from viruses, and interferon inducers are being studied in some viral illnesses. In general, prevention of viral illnesses through vaccination is the hope for the future.

Bacteria.—Many bacteria have been identified as the cause of diarrheal illness, and the list is growing as laboratory methods for identification improve. Other bacteria have been isolated from patients with and without diarrhea, and some of these are clearly pathogenic. In many areas, the facilities to identify etiologic agents of diarrhea are unavailable.

Most episodes of bacterial diarrhea are self-limited and specific. There are effective antibacterial agents for most of the pathogenic organisms, but these agents are frequently unavailable or too expensive. Alternatives in special situations (e.g., allergy to an agent or resistance) often are not readily available. Resistance to drugs by many enteric bacterial pathogens is growing.

Vibriocholerae.—Tetracycline has been the drug of choice for treating cholera, but it has several drawbacks. It causes staining of teeth in children, the group in which most diarrheal illness occurs. Tetracycline also sensitizes the skin to sunburn and is not recommended for pregnant women during the stages of bone and teeth development. Alternatives to tetracycline are furazolidone, chloramphenicol, and trimethoprim/sulfamethoxazole (T/S).

Cholera strains had remained almost universally susceptible to tetracycline until 1977, when 76 percent were found resistant after 5 months of an epidemic Tanzania (226). In December 1980, 18 percent of strains of *Vibriocholerae* isolated in Bangladesh were multiply resistant to tetracycline, ampicillin, kanamycin, streptomycin, and T/S (131). Some of these strains are able to transfer their resistance factors to *Escherichia coli* (131).

Shigella.—Many species of *Shigella* cause diarrhea. *Shigella* usually cause endemic diarrhea, but may cause epidemics. In general, effective agents such as ampicillin, chloramphenicol, and T/S are available to treat shigellosis, but there is a growing problem with resistance. Sulfonamide resistance was described in Japan in the 1950s and was described subsequently elsewhere (185). Areas which had previously had uniformly sensitive strains began to have consistently resistant strains.

Subsequently, strains were isolated that were resistant to more than one antimicrobial. Investigators discovered that these *Shigella* were probably acquiring resistance factors from other organisms present in the gastrointestinal tract, specifically *E. coli.* The transfer of resistance factors between bacilli has become an important complication of the treatment of many bacterial infections; some of these are mentioned later in this chapter.

The exact reason for the increase in the incidence of resistance factors in *Shigella* is not entirely clear, but is, in general, thought to result from the widespread use of therapeutic agents in a population, especially when they are used indiscriminately or in inadequate dosages. Such use frequently occurs when the agents are available over-the-counter, as they are in many developing countries. *Shigella* resistance not only is a problem in the developing world, but also has been reported in Washington, DC (294). Up to 93 percent of strains in some areas have been resistant to more than one drug (294).

Salmonella.—For many years, chloramphenicol has been the most reliable drug in the treatment of typhoid and typhoid-like enteric fever (infection due to nontyphi species of *Salmonella*). With the development of new antimicrobials, alternatives such as ampicillin, amoxicillin, amdinocillin, and T/S have become available.

Drug resistance in *Salmonella* was not much of a problem before 1972. Since then, there have been outbreaks of disease with resistant organisms in more than one country (128). Strains of both typhi and nontyphi species have been found to be resistant (30). More importantly, multiply resistant strains of *S. typhi* have been isolated. Occasionally, these strains are resistant to both cloramphenicol and ampicillin (38). More recently, strains resistant to chloramphenicol, ampicillin, and T/S have been isolated (188). Again, some of these resistant strains are able to transfer resistance factors to other bacteria (187).

Escherichia coli.—Antimicrobials are ineffective in decreasing the duration or volume of diarrhea associated with *E. coli* infection, but are sometimes recommended if the patient's diarrhea continues beyond 3 days or if the patient has bacteremia (bacteria in the bloodstream). Various strains of *E. coli* vary in their susceptibility to drugs, but they may be susceptible to ampicillin, chloramphenicol, and T/S.

Enterotoxigenic strains of *E. coli* (those that cause disease by a toxin produced by the bacterium) frequently cause diarrhea in travelers. A number of antimicrobials have been shown to be effective prophylactically including doxycycline, minocycline, trimethoprim, and T/S. Doxycycline-resistant strains have been isolated from Peace Corps volunteers in Thailand (102). Besides nausea, the possibility of skin photosensitivity from doxycycline and minocycline exist, although reactions to these agents are less frequent than those due to tetracycline.

Campylobacter.—The diarrhea resulting from *Campylobacter* infections frequently lasts only a few days, and therapy usually is not necessary. *Campylobacter* are generally susceptible to erythromycin, tetracycline, furazolidone, chloramphenicol, and clindamycin; erythromycin continues to be the drug of choice. In some areas, strains are resistant to tetracycline (113).

Other Causes.—Other bacterial causes of diarrhea are species of *Aeromonas, Pleisiomonas, Yersinia, Vibrio* (noncholera), *Staphylococcus, Clostridium difficile,* and *C. perfringens. Aeromonas* produces a toxin that causes diarrhea that is usually self-limited; it can usually be treated with erythromycin, tetracycline, and chloramphenicol, but is resistant to ampicillin. *V. parahemolyticus* is one of the noncholera vibrios which cause diarrhea. Treatment is usually unnecessary. *S. aureus* and *C. perfringens* are common causes of food-borne outbreaks of gastroenteritis which are self-limited, and no treatment is advocated. *C. difficile* is an important cause of antibiotic-induced colitis in developed countries, although the incidence of this disease is not known, since detection methods are not available. The preferred drug is vancomycin, but the cost of one course of therapy is $200 to percent 500, more than most citizens of developing countries can afford. Metronidazole, bacitracin, and cholestyramine are cheaper alternative agents.

Protozoa.—*Giardia lamblia* and *Entameba histolytica* are common causes of diarrhea worldwide, but are particularly prevalent in countries with tropical environments. Giardiasis usually responds to either quinacrine, metronidazole, or tinidazole, and these agents are fairly well tolerated in the doses required. Therapy is not always effective, however, and retreatment may be necessary.

Symptomatic and asymptomatic intestinal ameba infections (amebiasis) respond to a number of drugs that are given orally and are well tolerated. Amebic liver abscess responds to a group of drugs known as "imidazoles," which includes metronidazole and tinidazole, and to more toxic drugs such as emetine, dihydroemetine, and chloroquine. Metronidazole in high oral doses causes abdominal cramps, nausea, and bloating and therefore is not tolerated by some people. Tinidazole is better tolerated and as effective. In patients who may not tolerate metronidazole and tinidazole, emetine and dihydroemetine are alternatives. Emetine and tinidazole may cause many adverse effects, including abnormalities of the heart rhythm, however, and it is recommended that patients receiving either of these drugs remain hospitalized.

Four other protozoa should be mentioned as the cause of diarrhea: *Balantidium coli, Isospora belli, Cryptosporidium* spp., and *Sarcocystis* spp. Balantidiasis responds to tetracycline and paromomycin. There is little information available on the proportion of diarrhea in tropical populations caused by the other three, and data on the effectiveness of treatment are only now becoming available. Furazolidine, T/S, and pyrimethamine-sulfa have been reported to be effective.

Helminths.—There are a number of nematodes (roundworms) implicated as causes of diarrhea, including *Ancylostoma duodenale* and *Necatur americanus* (hookworms), *Capillaria philippinensis, Strongyloides stercoralis* (threadworms), and *Trichuris trichura* (whipworms). For many of these, treatment may not be recommended the worm burden is not heavy and the risk of reinfection, is high, as is often the case in developing countries. For most roundworms, effective, safe, short-duration therapy is available.

Hookworms can usually be treated with bephenium, pyrantel pamoate, levamisole, or mebendazole in one or two doses for 3 consecutive days. Frequently, however, only the more toxic drug tetrachloroethylene is available.

S. stercoralis usually responds to thiabendazole, but this is one drug which is toxic and frequently causes anorexia, nausea, vomiting, diarrhea, and vertigo. Furthermore, thiabendazole is not always effective. Mebendazole is better tolerated, but still less effective.

T. trichura infections were a problem until the advent of mebendazole in 1971; now over 80 percent of these infections can be cured by a twice daily course for 6 days. Other alternatives effective against trichuriasis are oxantel, flubendazole, and albendazole.

Infection with *C. philippinensis* is a continuing problem. Mebendazole and thiabendazole are sometimes effective after 20 and 30 days respectively.

Praziquantel has revolutionized the treatment of trematodes (parasitic flatworms), many of which are associated with diarrhea: *Fasciolopsis buski, Echinostoma itocanum,* heterophyids, clonorchids, opisthorchids, and schistosomes. Virtually all of these respond to praziquantel.

Summary

Most cases of diarrhea can be treated successfully by treating the patient's symptoms with ORT. ORT is of particular benefit to infants and children, who may otherwise die from the dehydration that accompanies bouts of diarrhea. There are also safe, effective drug treatments for most of the bacterial infections that cause diarrhea, but not for viral infections. Protozoa and helminths that cause diarrhea can usually be eliminated with drugs, but the drugs are often toxic and not entirely effective. In general, however, the worldwide impact of these specific treatments, even if applied universally and successfully, would not approach the worldwide benefit of ORT if ORT were universally applied.

Acute Respiratory Infections (ARIs)

ARIs due to bacteria or viruses are usually either the major cause of death or second only to diarrheal disease in tropical environments. They are a major cause of morbidity everywhere. From a clinical point of view, infections are divided between the upper respiratory tract infections (URTIs) and lower respiratory tract infections (LRTIs), though the causative agents overlap to some degree. URTIs are infections that occur in and around the teeth, gums, sinuses, throat, tonsils, epiglottis, middle ear, larynx, and trachea. The most important LRTIs result in pneumonias. In many tropical countries, the methods necessary to make the cause-specific diagnosis of respiratory infections are not available. Many areas in developing countries do not have bacteriology or serology, not to mention the more sophisticated facilities necessary for culturing and identifying viruses.

Viruses

Most of the URTIs in tropical countries appear to be viral in origin, usually caused by the same viruses caused by disease in temperate environments—rhinoviruses, coronaviruses, adenoviruses, Ebstein-Barr virus, cytomegalovirus, influenza, parainfluenza viruses, etc. Most of these infections are self-limited, and although there is some morbidity resulting in loss of workdays, there is little mortality in adults. In children, there can be acute mortality associated with the development of a cycle of infection and malnutrition.

Many of the viruses that cause URTI also cause LRTI. The viruses that may cause severe pneumonia and death are measles, respiratory syncytial virus, and influenza. These generally cause death from overwhelming pulmonary involvement and respiratory failure. In many localities, the oxygen therapy that frequently is necessary is unavailable.

Antiviral chemotherapy is still in its infancy. Amantidine and rimantadine are effective in reducing the incidence and severity of symptoms due to infection with influenza A if given prophylactically or within the first 48 hours after the onset of symptoms. These agents are fairly well tolerated, but they are generally unavailable in the developing countries and relatively expensive.

There is no treatment available for the other common viral causes of URTI. Enviroxime is an antiviral agent available in Europe but not in the United States, and evidently effective against rhinovirus, one of the causes of the common cold.

Bacteria

Upper Respiratory Tract Infections (URTIs).—Four bacterial causes of URTI deserve specific mention. One, *Corynebacterium diphtheriae*, causes diphtheria, an acute, life-threatening pharyngitis. Another, *Bordetella pertussis*, is the cause of pertussis (whooping cough). The treatment of diphtheria is with antitoxin and penicillin or erythromycin. Antimicrobials are not effective in changing the course of pertussis. Both of these diseases can be prevented by immunization.

Streptococcus pyogenes, a Group A streptococcus known best as the organism precipitating rheumatic fever, is a common cause of pharyngitis. The disease is responsive to penicillin and erythromycin, among other antimicrobials.

Haemophilus influenzae causes ear infections, bronchitis, sinusitis, infection of the epiglottis, and meningitis, primarily in children. Ampicillin has generally been the drug of choice, although T/S and tetracycline also may be effective. Many strains of *H. influenzae* are now resistant to ampicillin. The preferred drug for serious infections involving ampicillin-resistant strains is chloramphenicol.

Lower Respiratory Tract Infections (LRTIs).—Effective antimicrobials are available for most of the pathogens causing pneumonia acquired in the community, although the variety of drugs is usually limited. Hospital-acquired pneumonias (nosocomial infections) in developing countries, just as in the developed countries, are often caused by drug-resistant bacilli or *Staphylococcus aureus*.

Streptococcus pneumoniae.—*S. pneumoniae*, the cause of pneumococcal pneumonia, is one of the most common causes of bacterial pneumonia, affecting almost all ages in both tropical and temperate climates. Pneumococcal pneumonia continues to be a frequent cause of mortality in some groups of patients worldwide even though effective antimicrobials are usually available and even though these patients are treated (13). *S. pneu-*

moniae is generally responsive to oral penicillin, cephalosporins, tetracycline, erythromycin, or chloramphenicol, as well as other antimicrobials. Patients who are very ill must receive these drugs by injection.

In 1967, a drug-resistant strain of *S. pneumoniae* was isolated from a patient in Australia (147). Subsequently, relatively resistant strains have been isolated from many parts of the world, especially South Africa and New Guinea, and some of these strains are multiply resistant with relative resistance to penicillin, tetracycline, and chloramphenicol. Patients with these strains have not responded to the usual doses of penicillin (394).

Streptococcus pyogenes.—*S. pyogenes* is a cause of pneumonia as well as URTI and is frequently associated with complications such as empyema (infection between the inside of the chest and the outside of the lung causing a collection of pus in that site). It responds to penicillin, but hospitalization and parenteral therapy are often required, at least initially.

Staphylococcus aureus.—Staphylococcal pneumonia not infrequently complicates recovery from viral infections, especially in children. Hospitalization is recommended with parenteral administration of semisynthetic enzyme-resistant penicillins or cephalosporins for about 2 weeks. Antistaphylococcal antimicrobials are effective and generally available.

Haemophilus influenzae.—Besides causing ear infections and meningitis, *H. influenzae* may cause pneumonia, most frequently in children. Most strains are sensitive to ampicillin, chloramphenicol, T/S, and the newer cephalosporins. However, drug resistance is prevalent in some tropical environments. Some strains are resistant to ampicillin, but occasionally there is resistance to both ampicillin and chloramphenicol, the antimicrobials most commonly recommended for treatment (40).

Klebsiella pneumonia and Escherichia coli.—*K. pneumonia* and *E. coli* are less common than *S. aureus* or *H. influenzae* as causes of community-acquired pneumonia. These organisms are usually susceptible to aminoglycosides such as gentamicin. Aminoglycosides must be given parenterally and are toxic to the kidney. Frequently, aminoglycosides are not available in developing countries.

Pseudomonas pseudomallei.—This organism causes melioidosis, a spectrum of disease ranging from acute to chronic pneumonitis, soft tissue infection, and other manifestations. Most cases have been reported from Southeast Asia. Treatment consists of tetracycline, chloramphenicol, and/or T/S.

Yersinia pestis.—*Y. pestis* is the etiologic agent of plague. It may cause fulminant pneumonia, which is highly contagious. Streptomycin is the drug of choice. Alternatives may be chloramphenicol, tetracycline, or T/S.

Others.—Other less common kinds of bacterial pneumonias are associated with brucellosis, leptospirosis, anthrax, glanders, salmonellosis, typhus, and Q fever. These pneumonias are usually only one manifestation of a more generalized illness. If the diagnosis can be made, treatment is usually available.

Mycoplasma

Mycoplasma are bacteria-like organisms that usually cause URTIs and atypical pneumonia in adolescents and young adults. Tetracycline and erythromycin are effective therapy and usually do not have significant adverse effects.

Protozoa

Pneumocystis carinii is a protozoan which causes pneumonitis in immunosuppressed patients in developing countries, mainly in malnourished children. In the United States, it is common among patients with acquired immunodeficiency syndrome (more commonly known as "AIDS"). The drug of first choice, a combination of trimethoprim and sulfamethoxazole, or a sulfa with pyrimethamine is effective in about 75 percent of cases. The alternative, pentamidine, is not readily available and is not entirely effective either. It must be given parenterally and has some serious adverse effects.

Amebiasis can sometimes cause acute and chronic pulmonary disease, but usually responds well to oral metronidazole.

Fungi

There are a number of fungi that cause acute and/or chronic pneumonitis and pleural disease in tropical environments. Most are responsive to amphotericin B. Amphotericin is toxic to the kidney, must be given parenterally, and is frequently unavailable in developing countries. Amphotericin-induced kidney failure is difficult to manage without the aid of dialysis, which also is frequently unavailable.

Helminths

Most adult helminths in developing countries do not initially infect the respiratory tract, but cause disease if the larval forms migrate through the lungs. Most of the time, this stage of the infection is self-limited and does not need treatment.

Echinococcus granulosus and other related species are "tapeworms." The larval worms form cysts in various organs, including the lungs. There is no effective medical treatment for tapeworm infection of the lung. Recently, a new drug, albendazole, has been demonstrated effective in a small number of patients. *Paragonimus westermani*, a fluke that causes small pulmonary cysts, has recently been shown to respond to praziquantel. The eggs of *S. mansoni* and *S. japonicum* can end up in the lung, causing a buildup of fibrous tissue around them. There is no available treatment for this stage of the disease.

Summary

Drug therapy is available for most bacteria, but not viral ARIs. Even with treatment, however, mortality rates from ARIs are high, especially among children. The general state of good health of the population that keeps mortality from ARIs low in the United States and other developed countries, is probably the most important factor lacking in developing countries.

Arboviral and Related Viral Infections

The 80 or so arboviruses known to infect humans cause four basic types of clinical conditions. Two are generally benign and self-limited, consisting of: 1) fevers of short duration, with or without a rash; and 2) painful joints and rash of short duration. Complications can develop from either of these two conditions, but they are the exception. The two more serious clinical syndromes caused by arboviruses are: 1) acute central nervous system disease usually with inflammation of the brain (encephalitis), ranging in severity from mild aseptic meningitis to coma, paralysis, and death; and 2) hemorrhagic fevers, with extensive hemorrhaging, and associated with shock and high case fatality rates (liver damage and jaundice accompany these symptoms in yellow fever).

There have been no specific treatments available for any of these viral illnesses. Management of the more serious conditions is exclusively supportive, directed toward alleviating major symptoms and preventing spread. Correction of fluid and electrolyte imbalance, oxygen, and blood transfusions are the most common measures.

Immune therapy with monoclonal antibodies has been effective in protecting mice against *Sindbis* virus and arboviruses (311). Plasma containing antibodies to one hemorrhagic fever virus, given to patients before the eighth day of illness, reduced mortality from 16.5 to 1.1 percent. However, these patients had more frequent relapses (211). Similar results were obtained with immune therapy in monkeys experimentally infected with Machupo virus (211). Immune therapy and ribavirin, a broad spectrum antiviral, is effective in decreasing the mortality from Lassa fever in monkeys (172). Both are undergoing clinical trials in Sierra Leone (219).

SUMMARY

This chapter has briefly outlined the present therapy available for the selected tropical diseases covered in this report. For some diseases, there has been, and continues to be, effective therapy, although there is a growing problem of drug resistance in diseases ranging from *P. falciparum* malaria to leprosy and other bacterial infections. For other diseases, therapy is available, but quite toxic. For still others, such as most viral infections, no therapy is available at all. Although tremendous progress has been made, the deficiencies are great, and much work remains to be done.

Case Studies

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Case Study A.—Oral Rehydration Therapy for Diarrheal Diseases

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Summary

In the living conditions prevalent in the less developed world, characterized by a lack of potable water, sanitation, and refrigeration, the bacteria and other pathogens that cause diarrheal diseases are easily transmitted to young children by contaminated water, hands, and food. As a result, infants in less developed countries suffer on the average six to eight separate episodes of diarrheal disease per child.

The two major consequences of infant diarrhea are dehydration and malnutrition. Diarrhea causes a loss of body water and salts (electrolytes), which, if sufficiently copious and not adequately replaced, may result in dehydration. Approximately 1 of every 150 to 200 episodes of infant diarrhea results in severe, life-threatening dehydration. In the last decade, diarrheal dehydration was one of the major causes of infant mortality in virtually all less developed countries.

All episodes of infant diarrhea have nutritional consequences, in part because ill infants eat less and in part because the pathogens that cause diarrhea often diminish the ability of the small intestine to absorb nutrients. Repeated diarrheal infections within a short period of time can lead to clinically overt malnutrition.

Irrespective of the specific infectious agent causing diarrhea, the treatment of diarrheal dehydration is the same and involves the replacement of body water and electrolytes (salts in solution). In the 1950s and 1960s, this treatment was accomplished by intravenous rehydration—i.e., infusion of sterile water and electrolytes into the vein of an infant. Although intravenous therapy is very effective, it was poorly suited to use in the less developed world where it was most needed. The drawbacks of intravenous rehydration include its expense; the need for sterility of the fluids, tubing, and needles; the requirement for relatively sophisticated health workers to administer the infusion; and the difficulties of distribution and supply of all the materials.

In recent years, thanks to important contributions by a number of laboratory scientists, clinicians, epidemiologists, and health delivery experts, a simple, efficacious, technologically appropriate alternative to intravenous rehydration has become available—namely, oral rehydration therapy (ORT) using sugar/electrolyte solutions. An application of ORT other than for treating dehydration involves its use *early* in the course of infant diarrhea to *prevent* dehydration. The ingredients used in oral rehydration solutions are widely available, easily transported, and need not be sterile. Furthermore, sophisticated health workers are not required to administer ORT.

The efficacy of an oral glucose/electrolyte solution to promote sodium and water absorption was demonstrated in clinical intestinal balance studies by U.S. investigators in the Philippines in the early 1960s. By the end of that decade, clinical studies by U.S. physicians and their Bangladeshi and Indian collaborators had established that ORT could diminish by 80 percent the intravenous fluid requirements of adults with severe cholera. Similar studies were carried out in children with cholera and adults and older children with severe diarrhea not due to cholera.

Since 1977, investigators have carried out a number of clinical studies perfecting the use of ORT in noncholera infant diarrhea in many less developed areas of the world. These studies have examined modifications in oral rehydration formulas including the sugar, base, sodium, and potassium content; they have resulted in practical regimens for health workers to follow in orally rehydrating infants; they have demonstrated the efficacy of oral rehydration, irrespective of the etiology of the diarrhea; and they have defined the limitations of ORT.

Several methods have been devised for the preparation of simple sugar/salt solutions that are safe and can be prepared in the home. These simple solutions are just as effective as more complex balanced glucose/electrolyte solutions in stimulating sodium and water absorption by the intestine, though infants treated with such solutions have inadequate potassium replacement and can become potassium depleted, a state with adverse clinical consequences.

ORT has been perfected in recent years to the point where it represents one of the most powerful weapons in the armamentarium against disease in the less developed world. Increasingly, less developed countries are undertaking national diarrheal disease control programs in which ORT constitutes the keystone.

Many U.S. pediatricians were initially somewhat resistant to ORT. Through a series of recent seminars, reviews, and publications in American pediatric journals, however, information on the efficacy and advantages of oral rehydration has been widely disseminated.

The development of ORT illustrates how basic physiological and biochemical research can lead to a highly effective therapeutic modality and practical public health tool. Specifically, the observation that during diarrhea, the intestine maintains its ability to absorb glucose and that sodium and water absorption accompany glucose absorption provided the physiologic basis for ORT as a clinical tool.

Introduction

More than one-half of the world's population lives in less developed areas that are characterized by a lack of potable water, inadequate means for disposal of human fecal waste, and intense crowding in rudimentary housing. Under such conditions of poverty, underdevelopment and lack of education, the pathogenic bacteria, viruses, and protozoa capable of causing diarrheal disease in humans are readily transmitted to young children. Indeed, under such conditions, the hands that touch infants, the weaning foods that are fed to infants, and the water that infants drink are heavily contaminated with fecal material, and therefore with diarrhea-causing pathogens. As a consequence, in the first 2 years of life, young children in the less developed countries bear an enormous burden of diarrheal diseases.

This case study examines the causes of infant diarrhea, its consequences, the magnitude of the problem, and the interrelationship between infant diarrhea and other major public health problems of the less developed world. The primary focus of the case study is on ORT. The discussion that follows reviews basic scientific contributions that provide a physiologic basis for this therapy, obstacles that had to be overcome to give it widespread acceptance, and hurdles that are now having to be passed in order to realize implementation of ORT on a global scale.

Definitions of Infant Diarrhea and Dehydration

The term diarrhea stems from the Greek word meaning "to flow through" and denotes an increased number of stools per day which are in an unformed or liquid state. Since the daily stool patterns of infants vary greatly owing to diet, feeding frequency, etc., the most useful operational definition of infant diarrhea is when the mother (who best knows her baby's stool pattern) states that her child has diarrhea (71). Typically, a state of diarrhea is characterized by at least three loose stools within a 24-hour period. Since infancy in American pediatric terms refers to the first 12 months of life, strictly speaking, infant diarrhea would refer to illness within that period only. However, since diarrheal diseases remain an important cause of morbidity and mortality for at least the first 24 months of life in less developed areas, the term "infant diarrhea" in common usage has come to refer to the illness in children up to 2 years of age (71).

Seventy percent of the human body, by weight, consists of water. Approximately 55 percent of the body water is located inside tissue cells and is associated with potassium ion (K^+); the remaining 45 percent is extracellular and is associated with sodium ion (Na^+). Irrespective of the specific infectious cause of an episode of diarrhea, the common denominator from the pathophysiologic point of view is the loss of body water and salts (electrolytes) in the diarrheal stool. Diarrhea represents mainly loss of extracellular water and Na^+, but K^+ is also lost in significant quantities.

In the older child or adult, diarrhea is usually a nuisance rather than a mortal danger (although the copious purging that occurs with cholera can dehydrate even adults). In children under 2 years of age, however, diarrhea of any etiology is potentially life-threatening if stool losses are copious. The reason is that the losses of body water and electrolytes resulting from loose stools can lead to significant dehydration, which, in the face of continuing unreplenished losses, can result in acidosis, shock, and death. In virtually all the less developed countries of the world, diarrheal dehydration is either the first, second, or third most common cause of infant deaths (22,48,71, 121,159).

Infants are at enhanced risk of development of severe dehydration for several reasons:

- Per kilogram of body weight, an infant each day requires 2½ times more water than an older child or adult (67). This requirement is mainly due to the greater surface area of the infant per kilogram of body weight, which results in proportionally more water loss by transpiration through the infant's skin.
- An infant cannot talk to specifically communicate thirst and request fluids.
- An infant cannot walk to a source of water or fluids to help himself or herself quench thirst. The infant is thus totally dependent on its mother or caretaker. In many cultures across the world, mothers actually decrease the amount of fluids (and breast milk) offered to infants with diarrhea

in the incorrect belief that this will benefit the infant by "resting the gut."

- Certain homeostatic mechanisms of the kidney may be less efficient in the young infant, thereby diminishing the body's capacity to adjust successfully to physiologic derangements (3,6).

Etiology of Infant Diarrhea

Prior to the early 1970s, the etiology of most episodes of infant diarrhea was largely unknown, either in industrialized societies or in less developed areas (59,62,88,111,124,125,163,164,165). Since the early 1970s, however, there has been a veritable explosion of knowledge on the causes of infant diarrhea with many new agents being discovered.

The most important infectious agents that cause infant diarrhea are listed in table A-1. Currently, an etiologic agent can be identified in approximately 65 to 75 percent of the cases of infant diarrhea that occur in less developed countries if comprehensive microbiologic techniques are used (11,12,83,148).

Black and colleagues, working in Bangladesh, noted that two etiologic agents, rotavirus and enterotoxigenic *Escherichia coli*, were found in approximately 60 percent of the cases of diarrheal dehydration severe enough to be seen in a rehydration center (11). Black and colleagues also followed a cohort of infants prospectively in a rural Bangladeshi village (9). This more intensive surveillance detected mild as well as more severe cases of diarrhea, including cases that would not usually be seen in a treatment center because of their mildness. Enterotoxigenic *E. coli* was found to be the most common cause of diarrhea, accounting for three episodes per child per year. One-fourth of the episodes of *E. coli* diarrhea lasted longer than 10 days (9).

Magnitude of the Problem of Infant Diarrhea

In the United States, the infant mortality rate is 11 per 1,000 live births (i.e., 11 of every 1,000 babies born live fail to survive to their first birthday) (161), and diarrheal dehydration is an uncommon cause of death. In contrast, in less developed countries, infant mortality rates typically range between 100 to almost 250 deaths per 1,000 live births, and diarrheal diseases are almost everywhere 1 of the three major causes of infant deaths. In general, when the infant mortality rate exceeds 100 per 1,000 live births, at least one-third of all infant deaths are attributable to diarrheal diseases (32,101).

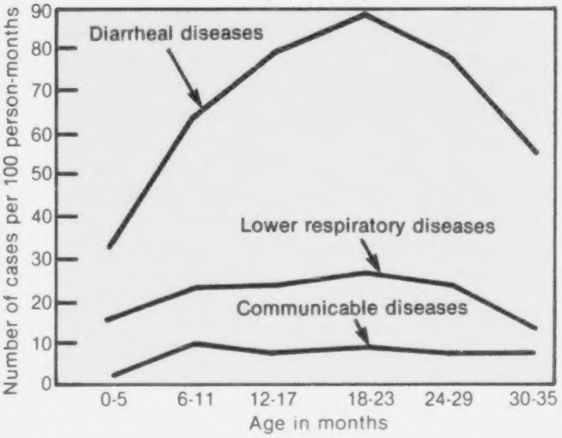
In the United States and other industrialized countries, infants experience one to two separate episodes of diarrhea per year (58,68). These episodes are usually mild, and because of easy access to health care, adverse consequences of the diarrheal illness rarely occur. In the less developed world, however, infants may experience six to eight separate episodes of diarrhea per year in the first 2 years of life (7,81,82). Results of a study of infectious disease incidence among children in less developed countries are summarized in figure A-1, showing the incidence of diarrheal disease to be much higher than rates for the other major causes of morbidity. Since each separate episode of clinical diarrhea lasts for several days, one calculates that infants in less developed countries spend approximately 15 percent of their entire life experience in the first 2 years, when rates are highest, suffering with diarrhea.

The World Health Organization (WHO) undertook to quantitate the magnitude of the problem of infant diarrhea in the less developed world by reviewing the published literature to determine the incidence rates of infant diarrhea and applying the calculated rates to the 1980 estimates for the population of children under 5 years of age (145). In an extremely conservative estimate, the investigators calculated that there are at least 750 million to 1 billion episodes of diarrhea and 4.6 million deaths each year due to diarrhea in children less than 5 years of age in Africa, Asia (excluding the People's Republic of China), and Latin America. They also estimated that approximately 1 in every 167 episodes of diarrhea in young children results in death.

Table A-1.—Infectious Agents That Cause Infant Diarrhea

Type of agent	Major importance	Lesser importance
Bacteria	Enterotoxigenic *Escherichia coli* *Campylobacter jejuni* Enteropathogenic *E. coli* *Shigella*	*Salmonella* *Aeromonas hydrophila* *Vibrio cholerae*
Viruses	*Rotavirus* Atypical adenoviruses	27NM Gastroenteritis viruses Astroviruses Calciviruses
Protozoa	*Giardia lamblia*	*Cryptosporidium* *Entameba histolytica*

Figure A-1.—Incidence of the Main Groups of Infectious Diseases Among Children During the First 3 Years of Life in Less Developed Countries (data from a cohort of 45 children observed from birth to 3 years of age)



SOURCE: Adapted from L. J. Mata, R. A. Kronmal, and H. Villegas, "Diarrheal Diseases: A Leading World Health Problem," in *Cholera and Related Diarrheas*, O. Ouchterlony and J. Holmgren (eds.) (Basel: S. Karger, 1980).

Nutritional Consequences of Infant Diarrhea

Besides dehydration, the other major consequence of infant diarrhea is malnutrition. Virtually every infectious agent that causes acute diarrhea results in a temporary state of intestinal malabsorption that may last up to several weeks, during which time ingested nutrients are not properly digested and absorbed (14,63,74,76,129). Furthermore, each episode of diarrhea has other nutritional consequences. The diarrheal infection itself, particularly if associated with fever, results in increased catabolism (i.e., increased breakdown of body mass to provide energy sources). Studies in Guatemala by Mata and colleagues (81,82) showed that infants and toddlers readily consumed the recommended daily caloric intake during periods when they were not ill. However, young children ill with diarrheal or respiratory infections simply did not consume the recommended intake of calories during the episode of illness. When episodes of illness occurred one after the other, children would fall off the normal growth curve and malnutrition would become evident. Another problem faced in many parts of the world is that mothers in many cultures will decrease offerings of food to infants with diarrhea.

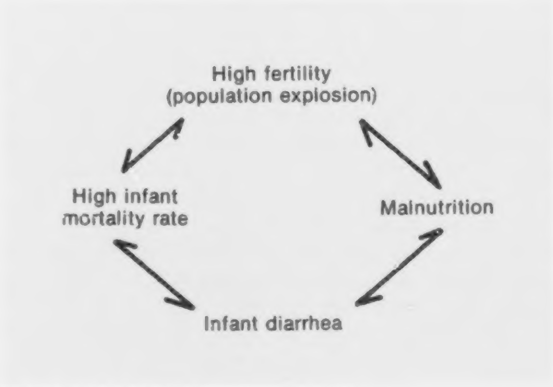
The Interrelationship of Infant Diarrhea With Other Major Public Health Problems of the Less Developed World

Certain health problems are common to all less developed countries. These include high infant mortality, diarrheal disease as a major cause of infant morbidity and mortality, high fertility (manifested as high population growth), and malnutrition (see fig. A-2). Because of interrelationships among these various health problems, control of some of them can have an important ameliorating effect on others. The interrelationships and the critical points of intervention are briefly discussed below.

The fertility rate of a country refers to the average annual number of live births per 1,000 women of childbearing age (15 to 49 years). High fertility leads to rapid population growth. One school of thought contends that high fertility is in great part the consequence of high infant mortality, since the latter creates compelling pressures for parents to produce more children in order to ensure survival to adulthood of some children (16,82,84). For example, in a particular culture, if two sons are believed to be required for the well-being and perpetuation of an agrarian family, the parents aim to produce a sufficient number of "insurance" children to ensure the survival of the desired number.

Multiple infant deaths and multiple pregnancies lead to the maternal deprivation syndrome—the poorly nourished, anemic, exhausted, women ubiquitously

Figure A-2.—Interrelationship of Factors Perpetuating Diarrheal Disease



SOURCE: Office of Technology Assessment, 1985.

encountered throughout the less developed world. The last infants of such women have even less chance of survival because of their mothers' diminished lactational capacity.

High fertility can lead to extraordinary population growth and pressure on land resources and limited food supplies. Some less developed countries (e.g., Kenya) have annual population increases of more than 3 percent. Consequently, the population is young (about 40 percent are less than 15 years of age) and doubles in size every 20 to 25 years!

There is an intimate relation between infant diarrhea and malnutrition (9,10,29,71,80,81,102,121,130, 143,152,154). Simply stated, multiple bouts of diarrhea lead to malnutrition. Malnutrition, in turn, predisposes to increased incidence, severity, and case fatality of diarrheal disease. Puffer and Serrano's (121) classic study of childhood mortality in the Americas documented the intimate correlation between malnutrition and death due to diarrheal disease

There is evidence that the malnourished child has more frequent bouts and increased severity of diarrhea. Protein malnutrition leads to decreased stomach acid (49); stomach acid is perhaps the most important nonspecific defense mechanism against bacteria that cause diarrhea. Diminished stomach acid allows inordinately large numbers of pathogenic bacteria to reach the small intestine.

Points of Intervention

How is it possible to intervene effectively to diminish or break the vicious cycle portrayed in figure A-2 and described above? In the past, attempts were made via nutritional intervention and family planning programs. Nutritional programs by themselves have largely failed, and family planning is least successful where infant mortality rates are high.

Some countries have broken this cycle in the course of just 25 to 30 years as a consequence of rapid capital development, industrialization and striking improvement in the general socioeconomic level. Just how rapidly these changes can occur is demonstrated by the New York City's experience between 1900 and 1930. Figure A-3 shows that the infant mortality rate in New York City in 1900 was 140 deaths per 1,000 live births, a typical rate for a developing country. The single most important cause of infant mortality was infant diarrhea (referred to as "cholera infantum"). By 1930, New York's infant mortality rate had plummeted to approximately 50 per 1,000 live births. This extraordinary fall in the rate was almost entirely attributable to the decrease in infant deaths from diarrheal disease. It is important to note that this decrease in New York's infant mortality from diarrheal disease occurred in the absence of modern treatments such as rehydration therapy or antibiotics and without the benefit of specific vaccines. Rather, the rapid improvement in living conditions (provision of potable water and sanitation systems) and in the educational level of the population resulted in a striking decrease in the incidence of diarrheal infections. As the population of New York City went from living conditions of underdevelopment to those of modern industrialized societies, the transmission of the micro-organisms that cause diarrhea in young children was sharply curtailed. Thus, both the incidence of diarrhea and mortality due to diarrheal diseases sharply decreased. The epidemiologic changes described in New York City were also experienced in European countries (including England, France, and Germany) at approximately the same time.

Although fundamental changes in quality of living conditions represent the optimal mode of intervention, such changes are unlikely to occur in many less developed countries in the near future because the means for rapid capital development and industrialization simply are not available. Other more practical, simplified interventions must be employed if they are to have an expectation of success in the short term.

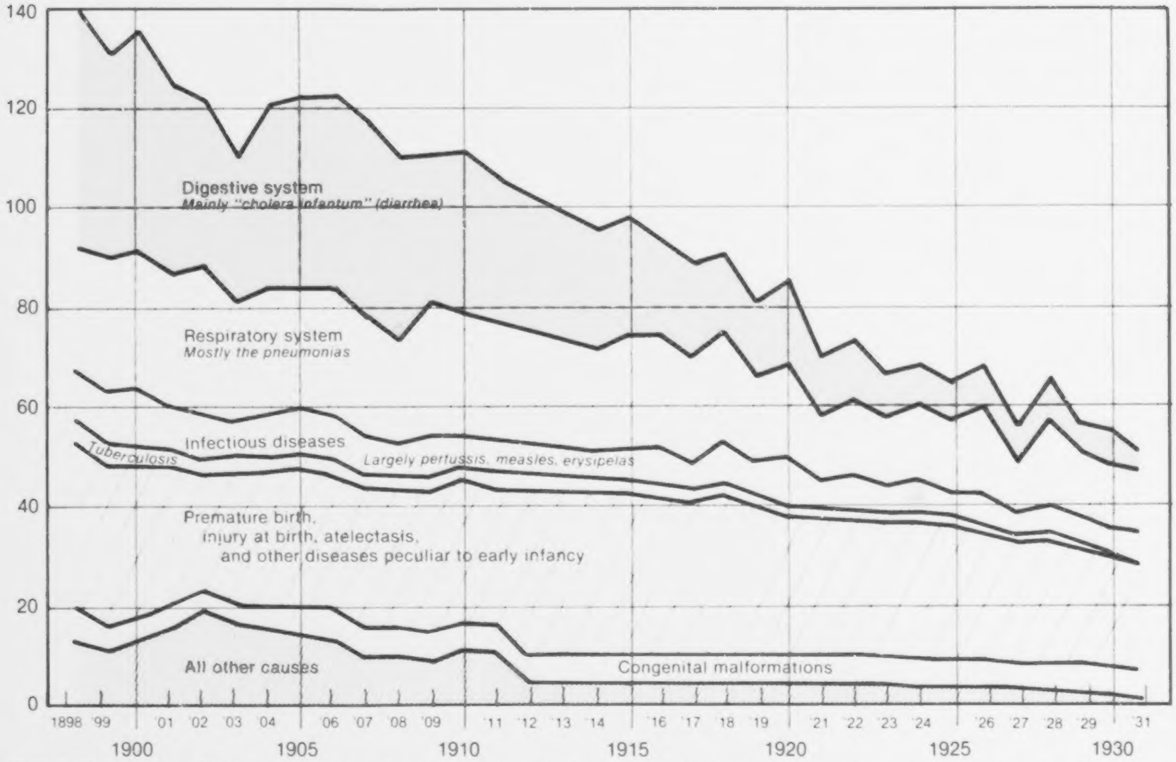
Up to the last decade, there was no simple and effective intervention to attack the vicious cycle in figure A-2 at the point involving infant diarrhea. However, such an intervention now exists: ORT. This is an inexpensive, highly effective means of replacing the deficits of body water and electrolytes in dehydrated infants,) that is technologically appropriate for use in less developed countries. ORT constitutes a revolutionary innovation in the treatment of diarrheal disease that has the potential to greatly diminish infant mortality throughout the world.

History of the Development of ORT

Intravenous Rehydration

Dehydration is the most important complication of diarrhea: untreated it can lead to death. An important therapeutic advance in pediatrics was the demonstration in the first half of the 20th century that deaths from diarrheal dehydration could be prevented if the dehydrated infant's deficits in body water and electrolytes were replaced by infusions into a vein of sterile electrolyte-containing solutions (54).

Figure A-3.—Infant Mortality by Prominent Causes in New York City, 1898-1930 (rates per 1,000 births)



SOURCE: W. McDermott, "Modern Medicine and the Demographic-Disease of Overly Traditional Societies: A Technologic Misfit," *J. Med. Educat.* 41 (Sept. Suppl.): 137-162, 1966, used by permission from the *Journal of Medical Education*.

This life-saving intravenous therapy gained widespread use in the United States in the 1940s, 1950s, and 1960s for the treatment of diarrheal dehydration in infants (54). Intravenous rehydration used by U.S. Navy physicians during large outbreaks of cholera in Egypt in 1947, in Thailand in 1958-60, and in the Philippines in 1962 dropped the case fatality rate for severe cholera from 20 to 50 percent down to 1 percent (108, 158,160).

Intravenous rehydration for the treatment of dehydrated infants also became increasingly popular in less developed countries in the 1950s and 1960s. However, its impact was limited because availability was generally restricted to hospitals in cities. Even in those facilities where it was used, it represented a significant drain on financial and personnel resources.

The disadvantages of intravenous rehydration include the following:

- Intravenous rehydration is expensive.
- The intravenous fluids and needles and plastic tubing required to administer them must be sterile; often these materials must be imported with hard currency.
- In general, a specially trained person is required to start the intravenous infusion, select the appropriate fluid, and supervise the volume and rate of administration.
- Use of the same needles and tubing in more than one patient without proper sterilization (a common practice in less developed areas) in an effort to decrease costs encourages the transmission of infections such as hepatitis from one patient to another.
- The mother is not directly involved in care of the infant.
- There are logistical problems in ensuring the delivery of supplies of intravenous fluids, tubing, needles, etc., to rural and less accessible areas.

Oral Rehydration—An Alternative

During the past 20 years, there has evolved an alternative form of therapy, ORT, that has multiple advantages over intravenous rehydration and is well-suited for use in less developed countries. This new therapy involves rehydration by the oral route using solutions containing glucose or sucrose (sugars) and electrolytes (salts in solution). The advantages of ORT include the following:

- ORT is inexpensive.
- The basic ingredients are found within most less developed countries.
- Sterile materials are not necessary.
- Highly specialized personnel are not directly required to administer the therapy.
- While therapy is in progress, the patient does not require the same degree of vigilance as a patient receiving intravenous rehydration.
- The mother can be directly involved in treatment by administering the oral rehydration solution to the infant.

Physiologic Basis of Oral Rehydration

The normal intestine has tremendous absorptive capacity. In the normal state in the healthy adult, 7 to 8 liters of endogenous fluids (including saliva, bile, stomach fluid, intestinal juice, pancreatic juice) are secreted into the intestine. In addition, approximately 2 to 3 liters of exogenous fluids are consumed each day. Yet at most, only 200 ml of water are lost each day in normal feces. The normal intestine absorbs 98 percent of the fluid that enters it in the normal state. In contrast, during diarrhea, the intestine exhibits net secretion.

The physiologic rationale for the efficacy of glucose/electrolyte oral rehydration solution is the fact that even during diarrhea, the simple sugar glucose continues to be absorbed by the upper small intestine (the jejunum) via an active transport mechanism (56,107,109). As molecules of glucose are actively absorbed, molecules of water and sodium (Na^+) are also absorbed; bicarbonate (HCO_3^-) and potassium (K^+) are absorbed during diarrhea even without glucose.

The first clinical balance studies documenting the importance of glucose as an actively transported substrate that promotes water and Na^+ absorption during diarrhea were carried out in 1962 in the San Lazaro Hospital in Manila, Philippines by Phillips and coworkers (108) of the U.S. Naval Medical Research Unit—2 (NAMRU-2), Taipei.

Prior to that time, several clinical investigators had published reports describing the use of glucose/electrolyte mixtures in oral rehydration of patients with diarrhea, but none of these investigators was aware of the critical importance of glucose in the absorption of Na^+ and water during diarrhea. Rather these workers had included glucose merely as a source of calories for the child with diarrhea. Darrow (34) and Harrison (44,52) used such solutions to treat infants with mild dehydration. Chatterjee (21) treated mild cholera patients with oral rehydration. The most extensive and impressive experience was reported by Menenghello and colleagues (86) from Santiago, Chile. Each summer during the 1950s, epidemics of infant summer diarrhea in Santiago overwhelmed the available health services. These Chilean workers set up oral rehydration units and rehydrated infants with a glucose/saline solution administered into the stomach through a nasogastric tube. In a comparative study, these Chilean workers noted that oral rehydration with their glucose/saline solution was as effective as intravenous fluids in rehydrating moderately dehydrated infants and was much more practical, simple, and economical. Menenghello and colleagues' experience was reported in the American pediatric literature in 1960 in the annual publication *Advances in Pediatrics*, but little notice was given to it.

Prior to 1962, there also had been several publications in the scientific literature establishing that in animal intestine glucose is actively absorbed and promotes the absorption of Na^+. Fisher and Parsons (45) are credited with being the first to show that glucose is actively absorbed by rat intestine and that its absorption promotes water absorption. Riklis and Quastel (127) in 1958, and Curran (31) in 1960, demonstrated that glucose also promoted the transport of Na^+ and Cl^- ions. Finally, Schedl and Clifton (140) as early as 1963, reported that glucose exhibited a stimulatory effect on the absorption of Na^+ and water by normal human intestine.

It is not clear whether Phillips and associates were aware of the above reports when they commenced their studies in Manila in 1962. Van Heynigan and Seal discusses this question in their book *Cholera—The American Scientific Experience 1947-1980* (155). They quote one associate of Phillips, Craig Wallace (p. 229), as believing that Phillips was indeed aware of these early physiologic studies reporting the effect of glucose on Na^+ and water absorption. In contrast, they point out that another friend and collaborator of Phillips, Sir Graham Bull (p. 229-230), was convinced that Phillips was unaware of the importance of glucose in stimulating absorption of Na^+ and that his inclusion of glucose in solutions given to certain patients in Manila in 1962 was "purely to make the solution isosmolar."

Whether Phillips did or did not know of the earlier studies is probably irrelevant in the historical context. What is important is that he carried out elegant and precise clinical balance studies on adult patients with cholera who ingested various solutions. In the course of these studies, he demonstrated for the first time the salutary effect of glucose on absorption of both Na^+ and water in patients with severe diarrhea.

Phillips first published his observations in 1964 (107). In these balance studies, Phillips measured diarrheal stool output (in ml/kg/hr) and quantitated the electrolyte content of the cholera stools. He also carefully measured the volume and rate of administration of the various oral solutions that he tested. By subtracting the hourly oral intake from the hourly stool output, Phillips was able to gauge the effects of various oral solutions on stool output.

In this way, Phillips clearly documented that pure distilled water, devoid of any electrolytes or other solutes, was readily absorbed by an adult cholera patient and was able to replace the water losses in moderate cholera. Phillips reported that the patient with cholera drank two glasses of water (500 ml) each hour without difficulty. However, choleraic stools of adults contain considerable Na^+ (approximately 135 mMol/l) as well as other electrolytes. Since plain water does not replace the Na^+ losses, patients with moderate cholera rapidly become deficient in Na^+. The Na^+ deficits must be repaired or the patient's life would soon be endangered.

Phillips had cholera patients drink a balanced oral solution that contained concentrations of electrolytes closely resembling those found in the stools of an adult with cholera. Phillips noted that in contrast to plain water, the electrolyte solution resulted in no absorption whatsoever of water or Na^+ even with the patient drinking 700 ml each hour. Only K^+ was readily absorbed. Phillips next demonstrated that when a healthy adult without diarrhea drank the same electrolyte solution at the same rate, no diarrhea occurred. This showed that the healthy intestine could easily absorb the water and electrolytes in the large volumes of the solution ingested.

In the next step, Phillips gave an electrolyte solution containing a much higher concentration of Na^+ (230 mMol/l) than cholera stool (135 mMol/l) or serum (140 mMol/l). Under these circumstances, as well, water and Na^+ losses increased in the stool.

Phillips next decided to "investigate the effect of oral solutions supplemented with nonelectrolytes such as D-glucose." The solution he used included 95 mMol/l of Na^+ and 100 mM/l of glucose. The patient drank the solution at the rate of 500 ml/hr. Phillips found that glucose was absorbed during cholera, and along with it water, Na^+, Cl^-, HCO_3^-, and K^+. Phillips exact words in the 1964 report are of interest (107):

> Furthermore, it was found that the glucose was absorbed, indicating that the glucose transport mechanism was not inactivated in cholera.

The use of the term "glucose transport mechanism" strongly implies that at least at the time of preparation of his 1964 manuscript, Phillips was aware of studies showing intestinal absorption of glucose by an active transport mechanism.

The last clinical experiment described by Phillips involved the evaluation of increasing concentrations of glucose from 50 to 400 mMol/l with the electrolyte concentrations remaining the same. As the glucose concentration increased, Na^+ losses in the stool decreased (i.e., Na^+ absorption increased).

Phillips' closing statement is prescient and worth noting in its entirety (107):

> *Oral therapy in cholera*. From the above studies it is evident that oral solutions can replace the H_2O, K^+, and some of the HCO_3^- losses in cholera. This means that the only intravenous requirement is the replacement of the Na^+ and Cl^- losses. We have further evidence which suggests that by incorporation of glucose in an oral solution that one may be able to develop an oral treatment regimen which in the average case might completely eliminate the requirement for intravenous fluids. I would like to urge caution on this particular point. Such a regimen can only be validated by careful balance studies of the type reported here. The literature on cholera abounds with treatment regimens which had been enthusiastically urged by their proponents in whose hands there has been great success. When the same treatment was tried by others, it appeared to be useless. I am sure this audience will readily understand why we believe it is necessary to carefully document any new therapeutic regimens suggested for the treatment of cholera.

Further Studies of Intestinal Absorption

In the 5 years following Phillips' 1964 report on the effect of glucose on intestinal absorption of Na^+ and water in patients with cholera, important research proceeded rapidly in two independent but mutually related areas. First, physiologists and gastroenterologists began to study intensively the precise mechanisms by which glucose enhanced Na^+ and water absorption in the intestine of healthy humans and animals (5,40,46,47,128,141,144). Second, concomitantly, Americans and Bangladeshis in Dhaka, East Pakistan (now Dacca, Bangladesh), and American and Indian investigators in Calcutta, India, carried out clinical intestinal flux studies to expand on and confirm Phillips' observations in the Philippines, again establishing the feasibility of using oral rehydration in the treatment of cholera (56,109).

Schultz and Zalusky (141) suggested that the enhancement of Na^+ transport by glucose is due to an interaction with the sugar transport per se, i.e., there occurs a glucose-coupled Na^+ transport. Sladen and

Dawson (144) came to the same conclusion from studies of intestinal absorption in humans. Fordtran and coworkers (47), who also studied glucose and Na^+ absorption in humans, concluded in 1968 that while a small fraction of Na^+ absorption occurs by active transport coupled to glucose, most Na^+ absorption occurs in the osmotic flow of water molecules created by the active absorption of glucose. (This means that as glucose molecules are actively absorbed, osmosis results in absorption of water molecules as well. And as the water molecules are absorbed, Na^+ is dragged along.) In a 1975 publication, Fordtran (46) reiterated that both active and passive sodium transport occurs in the jejunum and that the relative importance of each depends on the glucose and Na^+ concentrations in the jejunal lumen. Fordtran again concluded that solvent drag, rather than active cotransport, is the most important mechanism by which glucose stimulates net Na^+ absorption in the human jejunum.

Other investigators showed that substrates other than glucose, including certain amino acids, also promote the absorption of Na^+ and water as they are actively transported (50,128,142,147,151). This finding raises the possibility of including more than one actively absorbed substrate in an oral rehydration solution to stimulate absorption of water and Na^+.

Hirschhorn and colleagues (56), working at the Pakistan Southeast Asia Treaty Organization Cholera Research Laboratory (CRL) in Dacca, and Pierce and colleagues (109), working at the Johns Hopkins International Center for Medical Research and Training (ICMRT) at the Infectious Disease Hospital in Calcutta, carried out elegant clinical balance studies to expand the preliminary work initiated by Phillips (107) in patients with cholera. These clinical studies provided a sound physiologic basis in humans for proceeding with clinical trials of oral glucose/electrolyte therapy in cholera and other diarrheal diseases.

Hirschhorn and colleagues (56) infused various sugar/electrolyte solutions into the stomach or small intestine of eight patients with cholera. The electrolyte concentration of the solution was fixed (and included 134 mMol/l of Na^+), but varying concentrations of glucose, galactose, or fructose were added. Hirschhorn's group confirmed that glucose caused enhanced Na^+ and water absorption. Fructose, in contrast, was shown to have no enhancing effect.

Pierce and colleagues (109) carried out balance studies in 14 adults with cholera who were divided into three groups to receive one of three solutions by nasogastric tube. All three solutions contained approximately the same concentrations of K^+ and HCO_3^-, while the Na^+ and glucose concentrations varied. These investigators confirmed that the intestine's ability to absorb glucose remained intact during cholera and that glucose stimulated absorption of Na^+ and water. They reported that raising the glucose concentration of the solution infused into the stomach from 40 to 160 mMol/l doubled the absorption of water and Na^+. However, further raising the glucose concentration from 160 to 220 mMol/l did not further enhance water and Na^+ absorption.

Progression of Clinical Research Studies Leading to ORT as It Is Practiced Today

Early Studies in Adults With Cholera

The first publication investigating the therapeutic efficacy of an oral glucose/electrolyte solution was reported by Nalin and coworkers (95) from the CRL in Dacca and involved adults with moderately severe cholera. After receiving intravenous fluids to treat shock, 29 patients were randomly assigned to one of three groups. The first group of 10 patients continued to receive intravenous fluids, the standard therapy for cholera; the second group of 10 patients received a glucose/electrolyte solution by stomach tube after they were treated for shock with intravenous fluids; and the third group of 9 patients drank the glucose/electrolyte solution after they were successfully treated for shock.

Nalin's group (95) demonstrated for the first time that the intravenous fluid requirements of patients with severe cholera could be diminished by 80 percent if they either drank a glucose/electrolyte solution or had it infused through a stomach tube. The composition of the oral glucose/electrolyte solution used in this study contained 120 mMol/l of Na^+ and 110 mMol/l of glucose. Cholera patients drank 400 to 1,050 ml each hour without difficulty, and vomiting was not a problem.

In 1969, Pierce and coworkers (110) in Calcutta reported a similar favorable experience using an oral glucose/electrolyte solution in 20 adult patients with cholera. The solution contained 120 mMol/l of glucose but had a somewhat lower Na^+ concentration (100 mMol/l) than the solution used by the CRL group (95). Only one patient in the oral therapy group required some additional intravenous fluids to recover. The results of the Calcutta group corroborated the report from CRL.

In 1979, Cash and coworkers (17) reported from the CRL in Dacca that adults with moderate cholera and dehydration short of overt shock could be entirely treated by oral rehydration using a solution that contained 120 mMol/l Na^+ and 110 mMol/l glucose, completely obviating the need for intravenous fluids.

In other studies at this time, Nalin and Cash (93) compared glucose/electrolyte solutions containing 2 percent glucose (110 mMol/l) and 4 percent glucose (220 mMol/l), and found that net water balance was equally good with the lower concentration. Since Pierce and colleagues (109) had earlier shown that a solution containing 5 percent glucose offered no advantage over a 3 percent glucose solution, this combined experience led to the general acceptance of 2 percent glucose as the concentration in all future clinical studies with oral rehydration solutions.

Studies in Children With Cholera

Nalin's group at the CRL in Dacca next examined oral rehydration in children with cholera (94,96). In the early 1970s, the CRL investigators were rountinely using an oral glucose/electrolyte solution containing 120 mMol/l of Na^+ and 110 mMol/l of glucose. The results in children were as good as those obtained in adults with cholera. The intravenous fluid requirements of children with severe cholera were reduced by 80 percent, and children with mild or moderate cholera could be rehydrated entirely by the oral route. Evidence was rapidly accumulating showing the efficacy of oral glucose/electrolyte solutions in treatment of patients of any age with cholera.

Studies in Patients With Noncholera Diarrhea

The next major contribution of clinical investigators working in the Indian subcontinent was the demonstration that oral glucose/electrolyte solution was effective in treating adults and older children with diarrheal dehydration of etiology other than cholera (92,136). Most of these adult patients were later found to be infected with enterotoxigenic *E. coli* (137).

Preliminary Use of ORT in Field Rehydration Centers

Although experience with ORT in Dacca and Calcutta was rapidly increasing and the scope of its use was being expanded, ORT was at first used only in clinical research studies under carefully controlled conditions. Beginning in 1970, however, reports of evaluations of ORT under field conditions in rural treatment centers began to appear (18,78). Cash and coworkers (18) showed that ORT could be used successfully under field conditions in rural East Pakistan during an epidemic of cholera. As in the controlled environment, the requirement for intravenous fluids was decreased by 70 percent.

In 1975, a notable report by Bangladeshi researchers showed the promise of ORT (78) by describing its use in a crowded camp for Bangladeshi refugees during a cholera epidemic. Polyethylene packets of glucose and salts were prepared by hand. When mixed in 1 liter of water, the solution yielded 90 mMol/l of Na^+ and 121 mMol/l of glucose. No potassium was included since K^+ salts were unavailable. Under extremely adverse, primitive conditions, 3,703 patients with clinical cholera were treated with oral glucose/electrolyte solution and only a small supply of intravenous fluids. The case fatality rate was only 3.6 percent in this epidemic, largely because of the availability of ORT.

Early Reports of ORT in Infants With Diarrheal Dehydration

Despite the expanding use of ORT in Dacca and in Calcutta in the mid-1970s, there were few reports of its use in infants and young children outside of the Indian subcontinent. This represented a problem since the greatest need for oral rehydration worldwide was in the treatment and prevention of dehydration in infants and young children with noncholera diarrhea rather than in the treatment of adults with cholera.

Up through 1977, almost all of the reports describing oral rehydration of pediatric patients originated from Asia (almost all from the Indian subcontinent) and often included children with cholera. Thus, a false perception was perpetuated elsewhere in the world that oral therapy was mainly for cholera.

The earliest reports of the use of oral glucose/electrolyte solution outside Asia were those of Hirschhorn and coworkers (55,57), who treated moderately dehydrated Apache infants with oral rehydration in Arizona. In these pioneer efforts, the investigators clearly demonstrated ORT's efficacy by means of monitoring body weights, stool volume, and plasma protein concentration. The method of rehydration Hirschorn and colleagues described was referred to as "ad libitum" treatment, implying that when glucose/electrolyte solution is offered to an infant, the homeostatic mechanisms of the infant would lead the infant to drink until rehydration was established.

The "ad libitum" treatment regimen was not readily adopted by pediatricians or other health workers for three reasons:

1. The "ad libitum" treatment does not make use of the concept of estimation of fluid deficits and replacement of the calculated deficit. This is the fundamental physiologic concept on which all rational rehydration therapy, oral or parenteral, should be based (79).
2. The treatment is not easy to teach to unsophisticated health workers.

3. A small percent of overtly dehydrated infants do not readily drink oral rehydration solutions and must be actively coaxed. The fluid needs of such infants would not be recognized if a pure "ad libitum" method were followed.

Clinical Studies of ORT in Infant Diarrhea Since 1977

Since 1977, there has been a veritable explosion of information on the use of ORT in infants in many areas of the world including Central and South America, Africa, Southeast Asia, and the Caribbean basin. In careful clinical research studies, the remaining voids of knowledge were filled and obstacles impeding widespread acceptance of ORT were overcome. Much of this research was carried out by five groups: from the University of Maryland School of Medicine; the National Children's Hospital, San Jose, Costa Rica; the International Center for Diarrheal Diseases Research, Bangladesh (ICDDR,B); the Baltimore City Hospital; and the Kothari Pediatric Gastroenterology Center, Calcutta. Most of these studies in infants and children used the glucose/electrolyte solution recommended by WHO (shown in table A-2) or a very similar solution. These clinical studies established the efficacy of ORT in infants, identified its limitations, examined efficacy in relation to etiology, and evaluated modifications in the sugar and base content, and Na^+ and K^+ concentration in oral rehydration solutions. These studies are summarized below.

Clinical Indications, Regimens, and Limitations of ORT

Development of Practical Methods To Rehydrate Dehydrated Infants

Several groups of researchers have described regimens of ORT that were highly successful in rehydrating dehydrated infants (except those in overt shock) (24,28,100,114,139). These methods used the concepts of percent dehydration and fluid deficit estimations. Three methods (28,100,114) included offerings of plain water to infants, in addition to sugar/electrolyte solutions, to prevent the development of hypernatremia (a condition in which the serum Na^+ concentration exceeds 150 mMol/l).

Two groups (24,139) have demonstrated that dehydrated infants can be safely rehydrated with sugar/electrolyte solution alone without hypernatremia developing. Other investigators (113,132) have corroborated these findings.

Table A-2.—WHO Glucose/Electrolytes Solution

Ingredients:	
NaCl	3.5 gm
$NaHCO_3$	2.5 gm
KCl	1.5 gm
Glucose	20 gm
H_2O	1.0 liters

Electrolytes in resulting solution (mMol/liter):

Na^+	K^+	Cl^-	HCO_3^-	Glucose
90	20	80	30	111

Acceptability of Oral Solutions and Intestinal Water Absorption

Many researchers have documented that the vast majority of overtly dehydrated infants, despite fever, malaise, and lethargy, avidly drink sugar/electrolyte solutions (19,20,24,26,28,73,100,113,114,118,132,139), even though the taste is described as unpleasant by healthy adults. These overtly dehydrated infants gain weight from absorption of water and electrolytes (19,20,24,26,28,73,100,113,114,118,132,139). Approximately 95 percent of dehydrated infants, even those 10 to 12 percent dehydrated (but not in shock) can be successfully rehydrated with oral sugar/electrolyte solutions. (Dehydration with loss of more than 10 to 12 percent of body weight is almost always accompanied by clinical shock. Mild dehydration refers to up to 5 percent loss of body weight; moderate dehydration means loss of 6 to 9 percent; 10 percent or greater loss of body weight represents severe dehydration.) The demonstration that infants with 8 to 12 percent dehydration could be orally rehydrated was important, because it dispelled the contention of some pediatricians (101) that oral rehydration alone was incapable of rehydrating infants with moderate and severe dehydration short of shock.

ORT's Efficacy in Relation to the Etiology of Diarrhea

Studies from Costa Rica (100) and those of investigators in other parts of the world, including Dacca (13,134,150), have shown that ORT is effective in 95 percent of cases of diarrheal dehydration, irrespective of etiology. ORT is equally efficacious whether diarrhea is due enterotoxigenic bacteria (e.g., enterotoxigenic *E. coli*, *Vibrio cholerae*), invasive bacteria (e.g., *Shigella* spp., *Campylobacter jejuni*), enteropathogenic *E. coli*, or viruses.

Of particular interest is ORT's efficacy in rotaviral infection. Davidson and coworkers (35) showed that in isolated loops of piglet small intestine heavily infected with human rotavirus, there was no evidence for glucose-coupled Na^+ absorption. On the basis of these observations, they inferred that oral glucose/electrolyte solutions might be ineffective in infants with rotavirus infection. In fact, however, ORT is as effective in infants with rotavirus infection as it is in nonrotavirus infection (13,100,134,150).

The pathophysiologic explanation is that rotavirus causes patchy involvement of the small bowel with islands of denuded intestinal surface surrounded by normal or minimally affected areas. While the severely affected areas undoubtedly are defective in active glucose absorption, the nonaffected surface of the bowel absorbs sufficient glucose to permit excellent clinical results. When Nalin and coworkers (100) measured stool glucose concentration during oral therapy in infants with rotaviral diarrhea and infants with nonrotaviral diarrhea, the concentration was significantly higher in the former, demonstrating that indeed glucose absorption was somewhat impaired. Nevertheless, sufficient active transport of glucose occurred to allow 92 percent of rotavirus-infected infants to be successfully treated with ORT. In Costa Rica, rotavirus is by far the major single cause of diarrheal dehydration in infants (83), and ORT is the mainstay of therapy in the Emergency Room Service of the National Children's Hospital. Similar success in the treatment of dehydration due to rotavirus has been reported in Bangladeshi infants (13,150).

Oral Rehydration and Vomiting

Infants with diarrhea commonly manifest vomiting. Pediatricians and nurses unfamiliar with ORT often conclude that ORT cannot be instituted or that it will not be effective in those cases. This is a fallacy. In carefully monitored clinical studies in Costa Rica and Honduras (28,100,114), most of the infants had a history of vomiting on admission and most also experienced one or more episodes during ORT. However, careful balance studies in which the volume of vomitus was carefully recorded showed that with rare exceptions, the volume vomited was only a small fraction of the total volume of glucose/electrolyte solution ingested (28,73,100,114). From the perspective of the fluid and electrolyte status of the patient, it is not the frequency of vomiting that is of importance but the net balance of intake versus emesis (vomitus) and stool volume.

Studies in Costa Rica (114) have shown that vomiting tends to be more frequent during the early hours of ORT and diminishes thereafter. This observation suggests that emesis in some infants may be the consequence of electrolyte imbalance and acidosis.

The rate of emesis is also related to the sugar in the solution and to the method of administration. In comparative trials between glucose/electrolyte and sucrose/electrolyte solutions, children who received the sucrose/electrolyte solution had higher rates of vomiting (13,28). In summary, careful balance studies have provided the evidence to demonstrate that vomiting is rarely an obstacle to successful ORT.

ORT of Hypernatremic and Hyponatremic Dehydration

In approximately 70 percent of cases of diarrheal dehydration in infants, the concentration of Na^+ in serum remains normal (131 to 149 mMol/l). In a minority of cases, the serum Na^+ may be elevated (hypernatremia, ≥ 150 mMol/l) or abnormally low (hyponatremia, ≤ 130 mMol/l).

Hypernatremic diarrheal dehydration represents a serious complication. It usually occurs in young infants and can be accompanied by seizures, cerebral hemorrhages, and death. Hypernatremia is notoriously difficult to treat because of the propensity of infants to convulse during therapy. In the 1950s and 1960, hypernatremia was encountered in approximately 20 percent of infants with diarrheal dehydration seen in North America and Western Europe. Older American and European pediatricians and family practitioners, therefore, have great respect for this complication of infant diarrhea, consequent to their experiences several decades ago. In general, hypernatremia has been uncommon in infants in the tropics.

Some pediatricians have expressed concern that oral glucose/electrolyte solutions of the type recommended by WHO contain a Na^+ concentration (90 mMol/l) that is too high for infants, particularly those in industrialized countries (6,9,64,153,156,157). They suggest that in these well-nourished, predominantly formula-fed infants, who suffer mainly from rotavirus diarrhea, glucose/electrolyte solutions with Na^+ concentrations of 90 mMol/l are potentially unsafe and could lead to or exacerbate hypernatremia.

Considerable confusion has resulted from discussions on this point because of the failure to differentiate clearly between the use of oral glucose/electrolyte solution to *replace* fluid deficits in overtly dehydrated infants versus administration of glucose/electrolyte solution early in the course of diarrhea to *prevent* dehydration. The use of solutions with Na^+ concentrations of 90 mMol/l in nondehydrated infants with diarrhea must be accompanied by equal volumes of low solute fluids or plain water to allow the kidneys to handle Na^+ loads which are greater than the stool Na^+ losses. However, in the overtly dehydrated infant, even if hypernatremia is already present, there exists a total body *deficit* of Na^+ as well as water. In overtly dehydrated infants, a glucose/electrolyte solution containing 90 mMol/l is physiologically sound and clinically safe; indeed, it is an ideal rehydration solution to replace deficits (26,113,115,119).

Pizarro and coworkers have carried out several studies (113,115,119) in Costa Rica clearly establishing the safety of ORT of infants with hypernatremic dehydration. The problem with treatment of hypernatremic dehydration, intravenous or oral, is the occurrence of seizures (15,42,65,75,119). The last report by Pizarro and coworkers (113) described the use of slow oral rehydration in 35 infants with no episodes of seizures during therapy.

ORT also has been shown successful in treating infants with hyponatremia (serum Na^+ concentration ≤ 130 mMol/l) (113,119,139).

Experience With ORT in Neonates

At the National Children's Hospital in Costa Rica, the use of ORT in neonates has been exhaustively studied by Pizarro and colleagues (116,117). An extraordinary collective experience involving several hundred newborns documents that ORT is as safe and effective in this age group as in older infants. Furthermore, neonates with hypernatremic and hyponatremic dehydration can be as successfully rehydrated as older children (116,117).

Rapidity of Oral Rehydration

Complete replacement of the water and electrolyte deficit, accompanied by clinical signs of normal hydration and weight gain, is complete by 6 hours after beginning ORT in approximately 60 percent of cases; by 12 hours in 90 percent; and in 95 to 98 percent of instances by 18 hours (24,28,73,100,114,118). At the point of successful rehydration, infants are switched to the maintenance phase of rehydration, which includes the introduction of soft foods. Depending on social factors, the infant may be sent home at this point after the mother has been instructed in how to provide maintenance fluids at home and has been given packets to prepare the solution (118).

Limitations of ORT

Even in highly experienced units such as those in Costa Rica, Honduras, Bangladesh, and India, 2 to 5 percent of overtly dehydrated infants will fail on ORT alone. The most frequent causes of failure include the following:

- **Glucose or sucrose intolerance.** Overtly dehydrated infants often are severely malnourished and have intestinal bacterial infections. When given sugar/electrolyte solutions, these infants greatly increase their purge rates. This situation is seen in perhaps 1 percent of cases in less developed areas.
- **Intractable vomiting.** Occasionally, a dehydrated infant will simply be unable to drink without vomiting virtually the entire fluid volume that was just ingested. Such instances are rare.
- **Continuing high purge rates.** Most infants with diarrheal dehydration have diminished purge rates following replacement of fluid deficits or purge at rates that can be adequately replaced orally. Some infants, however, particularly those with cholera, continue to purge copiously at rates that cannot be easily replaced with ORT.
- **Abdominal distention.** Paralytic ileus or abdominal distention occasionally occurs, precluding continued ORT.

ORT is labor-intensive. Someone must continue to administer the fluids to the infant. If the mother or another guardian is not available, this requirement becomes a limitation. ORT units are dependent on the full cooperation of the infant's mother or a surrogate.

Composition of Oral Rehydration Solutions

Comparison of Glucose and Sucrose

Because of the lesser expense and greater availability of sucrose (table sugar), trials have been carried out to compare the efficacy of sucrose/electrolyte versus glucose/electrolyte solutions (13,99,103,134, 135). Sucrose molecules are "double sugars" twice the size of glucose molecules. For sucrose to be effective, it must be broken down by intestinal enzymes to its single sugar constituents, glucose and fructose, whereupon the actively absorbed glucose molecules promote water and glucose-coupled Na^+ transport. Fructose, in contrast, is not avidly absorbed and thus exerts a "solute drag" osmotic effect (56). In summary, the comparisons show that glucose/electrolyte solutions are slightly superior to sucrose/electrolyte solutions. However, the differences in overall efficacy are minimal so that if economic or logistic considerations are paramount, sucrose-based solutions can be routinely used with expectation of excellent clinical results.

Comparison of "Low" and "High" Sodium Rehydration Solutions

Several controlled studies have been carried out in which dehydrated children were rehydrated orally with either "high" Na^+ (90 mMol/l) or "low" Na^+ (50 to 60 mMol/l) glucose/electrolyte solutions (4,20, 98,133,138,139). High- and low-sodium rehydration solutions appeared equally efficacious in replacing fluid deficits.

In a study in Jamaica (98), Nalin and coworkers detected transient hypernatremia in 4 of 25 minimally dehydrated infants who were given the WHO glucose/electrolyte solution without free water. The hypernatremia was mild (150 to 156 mMol/l) and was not associated with any adverse clinical signs or symptoms. Chatterjee and colleagues (20) in India reported similar results. Two of their dehydrated infants who had normal serum Na^+ concentrations on admission developed asymptomatic mild hypernatremia during ORT with a glucose/electrolyte solution containing 90 mMol/l. In contrast, Santosham and colleagues working in Panama and in the United States did not encounter hypernatremia (138,139) in their orally rehydrated infants who also received the solution without free water.

In the study in Jamaica (98), net Na^+ absorption was significantly less in the infants who received the low Na^+ (60 mMol/l) solution and hyponatremia developed in 3 of 31 infants. Furthermore, several infants who were hyponatremic on admission remained so during oral rehydration with the low Na^+ concentration solution. A comparison of "high" and "low" Na^+ sucrose/electrolyte solutions by Saberi and Assaee (133) in Iran gave comparable results as seen in the glucose/electrolyte comparisons.

In summary, while in "low" and "high" Na^+ oral glucose/electrolyte solutions give equivalent results in most dehydrated infants, the high Na^+ solution is safer for hyponatremic and hypernatremic infants.

Optimal Potassium Concentrations

Losses of potassium ions (K^+) in diarrheal stool can be significant, particularly in infants in less developed countries who have repeated episodes of diarrhea. In such infants, insufficient replacement of K^+ losses can lead to total body K^+ depletion, accompanied clinically by muscle weakness, ileus, cardiac arrhythmias, and hypokalemic kidney disease. Nalin and colleagues (98) compared K^+ balance in mildly dehydrated infants treated with the WHO oral rehydration solution (which contains 20 mMol/l K^+) versus a modified solution containing 35 mMol/l K^+. Net K^+ absorption at 24 hours was more than twice as high in the infants that received the solution containing the higher K^+ concentration (35 mMol/l), and none of these infants had abnormally low or high potassium blood levels at 6 or 24 hours after beginning ORT. In contrast, low potassium levels were detected in about one-fifth of the infants treated with the low K^+ solution. Based on these studies in Jamaica, the formula of the oral rehydration solution used in Costa Rica was modified to provide more K^+.

Acetate or Citrate in Place of Bicarbonate

The shelf life of optimally prepared glucose/electrolyte packets containing HCO_3^- is 3 years. However, the shelf life of poorly made porous packets is limited because of the discoloration and caramelization that occurs when moist glucose and bicarbonate mix. Sodium acetate and sodium citrate are being studied as substitutes for sodium bicarbonate to see whether they prolong the shelf-life. In the body, acetate and citrate are converted to bicarbonate. Pizarro and colleagues (112) and Patra and colleagues (105) recently have carried out double-blind randomized comparisons of glucose/electrolyte solutions containing citrate or acetate, respectively, versus the standard solution containing bicarbonate. The solutions with citrate or acetate were equally efficacious.

"Super Solutions"

In 1970, Nalin and colleagues (97) reported results of a clinical study in which a glucose/electrolyte solution was compared with an electrolyte solution that contained the amino acid glycine as well as glucose. Both glucose and glycine molecules are actively transported, but by different mechanisms. The objective of this study was to determine if a solution containing two distinct actively transported substances would result in faster and increased water and electrolyte absorption than a solution containing only glucose and electrolytes. Nalin and coworkers observed that the glucose/glycine/electrolyte solution was superior to the glucose/electrolyte solution in promoting water and Na^+ absorption in patients with severe diarrhea. In fact, the glucose/glycine/electrolyte solution was so much better that the duration of diarrhea and total stool volume were significantly diminished in patients who received the solution as compared to the group who received glucose/electrolyte solution.

The implications of this important observation were not widely appreciated at the time. Many public health authorities believed that the lesser availability of glycine and the increased cost of solutions having both glucose and glycine ruled against widespread use of such solutions. Since 1982, however, the concept of "super solutions" with more than one substance actively transported by the gut has reappeared with increased popularity (2,77,144). Patra and coworkers (144) in Calcutta compared glucose/electrolyte and glucose/glycine/electrolyte solutions in oral rehydration of infants with diarrheal dehydration. Their results corroborated those in adults with cholera 15 years earlier. The duration of diarrhea and the total stool volume significantly diminished in infants given the solution containing both glucose and glycine.

Work is under way to carry these investigations further. The goal is to develop a "super solution" containing up to three actively transported substrates that not only replaces water and electrolytes but actually diminishes diarrheal stool volume to the point where losses are no longer clinically relevant (2).

Some groups of investigators have been examining naturally occurring complex substrates as opposed to pharmaceutically prepared formulas in making of "super solutions" (90,106,162). They hope in this way to accomplish the same goals at lesser cost and with greater availability. Most work in this area uses rice powder (30 to 50 gm per liter) with the same electrolyte concentrations as in the WHO solution. Rice contains polymers of glucose (glucose molecules strung together in a chain) which can be broken down in the small intestine to single glucose molecules (90). Rice also contains glycine (30 to 36 mg/100 gm of rice). Clinical studies have shown rice powder/electrolyte solutions to be at least as effective as glucose/electrolyte solutions in oral rehydration of infants with diarrheal dehydration (90,106). Some studies in infants (106) demonstrate a clear superiority of a 5-percent rice powder/electrolyte solution over the glucose/electrolyte solution, with the former significantly lowering stool output, duration of diarrhea, and intake of oral rehydration fluid.

ORT To Prevent Dehydration

Up to this point, ORT has been discussed in this case study as a substitute for intravenous rehydration to treat 95 percent of infants with overt clinical dehydration. Used this way, ORT is practiced by health care providers in health care facilities.

The second major use of ORT is to initiate the use of oral sugar/electrolyte solutions early in the course of diarrhea in an attempt to *prevent dehydration*. Ideally, this intervention is carried out with a balanced, physiologically sound solution containing appropriate concentrations of K^+ and HCO_3^- (or other base) in addition to Na^+ and Cl^-. In infants, stool Na^+ concentrations in noncholera diarrhea usually range from 25 to 70 mMol/l, with a mean of approximately 45 to 50 mMol/l (89,100,101,149). Thus, a glucose (or sucrose)/electrolyte solution containing 40 to 50 mMol/l of Na^+ is ideal to replace ongoing diarrheal losses early in the course of diarrhea in young children (43,44). The WHO solution can also be utilized to replenish ongoing diarrheal losses in infants but in this instance, an equal volume of low solute fluids (or plain water) is given following each volume of glucose/electrolyte solution to provide free water to handle Na^+ loads.

Simple Sugar/Salt Solutions

It is not possible economically or logistically to provide a packet of balanced sugar/electrolyte powder to treat every episode of diarrhea in all young children in developing countries. For that reason, some observers have advocated the use of simple rehydration solutions of table salt and sugar, prepared and administered in the home (15,53,66,70,85,91,120). Depending on the purity of the sugar and salt, simple solutions may contain only Na^+, Cl^-, and sucrose.

Two major obstacles had to be overcome before the use of home-made simple sugar/salt solutions could be advocated with confidence. First, it was necessary to identify simple, technologically appropriate methods for producing solutions containing safe and effective levels of Na^+ and sucrose. This was regarded as critical by many authorities who feared that improper mixing could result in solutions with unacceptably high Na^+ and sucrose concentrations, which could conceivably induce hypernatremic dehydration, accompanied by convulsions, intracerebral hemorrhage, and high case fatality. Second, it had to be shown to what degree simple sugar/salt solutions promoted water and Na^+ absorption, how well they combatted acidosis (since they lacked base), and what clinical and biochemical problems might accrue from the lack of K^+ in the fluids.

Many methods for measuring sugar and salt in preparation of simple sugar/salt solutions have been described. Some methods, such as the "finger pinch of salt and fistful scoop of sugar" technique, use the human hand for measurement (25,91). Other methods make use of a teaspoon or bottle cap, and a glass or a 750 ml or 1.0 liter bottle (30,38,69,70,72). Some authorities favor the use of a special double-ended plastic spoon with one trough for measuring salt and another for sugar (79,53). There is great controversy over the reliability of some of these methods (8,23,25, 30,32,33,38,51,53,66,69,70,72,85,91,120,126,146). Many health workers consider certain methods such as the "pinch and scoop" inherently so variable that they are unacceptable (32,69,72). Others disagree and believe that the simplicity of the pinch and scoop method justifies its use (25,91,146). A large rural ORT program in Bangladesh (Bangladesh Rural Advancement Committee) makes use of the pinch and scoop method (33).

Despite controversy over particular methods of preparation of simple sugar/salt solutions, all involved parties appear to agree on two fundamental points.

The first is that one must adapt methods of preparation of simple sugar/salt solutions to local conditions using local utensils. The second is that the more supervision and teaching involved, the greater the reliability of the method and the greater the safety of the resultant solutions.

Simple Sugar/Salt vs. Glucose/Electrolyte Oral Rehydration Solutions

Although many health authorities avidly supported the use of simple sugar/salt solutions and many national diarrheal disease control programs relied heavily on them, prior to clinical studies in Honduras (28), there were no physiological data to support their efficacy or show their limitations. Clements and coworkers (28) treated 61 Honduran infants 3 to 18 months of age with diarrheal dehydration with either a simple sugar/salt solution (60 mMol/l Na^+, 3.0 gm percent sucrose, no K^+ or HCO_3^-) or with the WHO glucose/electrolyte solution. The simple sugar/salt solution was found to be equal to the glucose/electrolyte solution in stimulating Na^+ and water absorption and almost as good in combatting acidosis. However, because of the lack of K^+ in the simple sugar/salt solution, significantly more infants treated with this solution developed abnormally low serum K^+ concentrations. Small amounts of banana puree were unable to replace sufficient K^+. It would require at least 320 ml of banana puree (280 ml of mashed banana, 2 to 3 whole bananas) to provide as much K^+ as contained in the amount of WHO glucose/electrolyte solution given over 24 hours to an infant with diarrhea (27).

In summary, properly prepared simple sugar/salt solutions are highly effective in promoting water and Na^+ absorption, thereby restoring a normal blood volume and blood flow through the kidneys which combat metabolic acidosis. However, the lack of K^+ in most simple sugar/salt solutions represents a critical drawback which can lead to abnormally low serum K^+ concentrations. Until the problem of adequate K^+ replacement can be resolved, simple sugar/salt solutions must be regarded as suboptimal alternatives for use only where a balanced sugar/electrolyte solution is unavailable.

Effect of ORT on Nutritional Status

A study in the Philippines showed that children who received ORT with glucose/electrolyte solution during bouts of diarrhea gained significantly more weight in ensuing months than matched control children who did not receive ORT (60,61). Studies in two other countries (Turkey and Iran) had similar results (7,39). Field workers, in the Gambia, however, have not been able to corroborate the results (131). One possibility is that ORT stimulates the appetite of children with diarrhea, and this accounts for the increased weight gain. Since early reintroduction of feeding is part of the ORT intervention, it is difficult to determine what role ORT per se plays with respect to food consumption. More information about ORT's effect on food consumption and nutritional states is needed.

Effect of ORT on Infant Mortality

Implementation of ORT programs in less developed countries would be expected to diminish diarrhea-related infant mortality. Unfortunately, however, few epidemiologic data are available to demonstrate this effect. Reports from India, Bangladesh, and Egypt provide some insights, but none is completely convincing because of methodologic problems (1,66,123). More data on this important question need to be gathered.

Dissemination of ORT

Many international, national, religious, and private agencies are involved in disseminating the use of ORT throughout the world. The most important agencies are the following:

1. World Health Organization (WHO),
2. Pan American Health Organization (PAHO),
3. U.N. International Children's Emergency Fund (UNICEF), and
4. U.S. Agency for International Development (AID).

World Health Organization

ORT is the keystone in WHO models for national diarrheal disease control programs.

WHO's Diarrheal Disease Control Program has played a pivotal role worldwide in stimulating the use of ORT. This has involved:

- provision of treatment guides, manuals, etc., in several languages;
- provision of technical consultants to countries;
- assistance in organization of national and regional workshops on ORT and diarrheal disease control; and
- provision of research grants to support basic, clinical, and operational research related to ORT.

Pan American Health Organization

PAHO is both the WHO regional office for the Americas and the Pan American Sanitary Bureau. Both independently and in conjunction with WHO, PAHO has pursued dissemination of ORT. This has involved:

- provision of technical consultants;
- support of national and regional meetings. PAHO has been active in promoting workshops on diarrheal diseases and ORT; and
- provision of research grants, equipment, and materials to clinical and public health investigators.

In the late 1970s, the first generation of PAHO ORT consultants were mainly North Americans. These consultants trained a second generation of PAHO ORT consultants from Latin America and the Caribbean who have since become internationally recognized authorities.

U.N. International Children's Emergency Fund

UNICEF has been a major supplier of packets of glucose/electrolyte powders to allow countries to start national pilot programs. UNICEF also has supplied some countries with machines to package their own ORT powders. ORT is an integral part of "GOBI/FF" program (growth chart, oral therapy, breast feeding, immunizations/family planning, female literacy).

U.S. Agency for International Development

AID has made major contributions to the dissemination of ORT. Some of the most important contributions include:

- provision of technical consultant support,
- support for meetings and workshops, and
- special projects.

The AID-supported international meeting on ORT in Washington, DC,in June 1982 was an important instrument to disseminate ORT. One of AID's special projects is the Mass Media Diarrhea Project, which has pilot programs in Honduras and the Gambia. Mass communications techniques (mainly radio) are being used to spread health information about diarrhea, dehydration, and ORT. The effectiveness of this program in Honduras is very impressive. Another special AID project has been direct financial support for the International Center for Diarrheal Diseases Research, Bangladesh. This is the offspring of the CRL (Cholera Research Laboratory), which has played an important role carrying out basic, clinical, and operational research on ORT.

Attitudes of U.S. Pediatricians Toward ORT

The vast majority of pediatricians practicing in the United States were trained in programs where the following attitudes were fostered:

- ORT helps to prevent dehydration and is useful in treatment of mild dehydration.
- ORT solutions for U.S. children should not contain more than 60 mMol/l of sodium in order to preclude the development of hypernatremia (a much feared complication).
- Children with moderate or more severe dehydration must be given intravenous rehydration.
- If a child is sufficiently dehydrated to receive intravenous fluids, nothing further should be given by mouth for 24 hours. The bowel should be "rested."

These have been the fundamentals of diarrhea therapy taught to two generations of American pediatricians.

Because of the large number of reports on ORT that have been published in recent years in the most prestigious pediatric journals in the United States (*Journal of Pediatrics, Pediatrics,* and the *American Journal of Diseases of Children*) and Britain (*Archives of Disease in Childhood*), most U.S. pediatricians are aware of the expanded use of ORT. Most are also cognizant of the composition of the WHO oral rehydration solution and accept its use as an important advance for the treatment of diarrhea in the less developed world. Some pediatric training programs in the United States have adopted ORT and use the WHO (as well as other) rehydration solutions. Throughout the United States, however, there remain many pediatricians who are skeptical of ORT and who are particularly resistant to use of a formula like the WHO glucose/electrolyte solution which contains 90 mMol/l of sodium.

The American Academy of Pediatrics has played an important role in fostering information on ORT and in attempting to forge agreement on the role of solutions containing 90 mMol/l of sodium in treatment of diarrhea in the United States. At its autumn national meeting in New York City in October 1982, the Academy convened a workshop on ORT. Other presentations on oral rehydration were made at American Academy of Pediatric meetings in May 1983, in Philadelphia, and in October 1983, in San Francisco.

In February 1983, the Academy convened a task force on ORT which met in New York. The assignment of the task force was to prepare recommendations on the following issue:

> . . . the advantages and disadvantages of Oralyte, a high electrolyte solution (distributed worldwide by UNICEF/WHO) in the treatment of infants with diarrhea and dehydration.

Initially, the task force was not able to come to an agreement. Most of the task force members wanted to make Oralyte (the WHO solution) a prescription drug in the United States for fear that some infants would develop hypernatremia (abnormally high serum Na^+ concentrations) if it were used without medical supervision. One dissenter argued that Oralyte had been proved safe worldwide and was safer than aspirin (which is sold without prescription). Some task force members believed that adopting a prescription requirement for Oralyte in the United States could have serious repercussions internationally, perhaps impeding the dissemination of in less developed countries.

A compromise was accepted for the task force to prepare recommendations, which were sent to the Academy Committee on Nutrition and then transmitted to the Food and Drug Administration to assist that agency in developing policy and regulations. The major points of the compromise include the following (28a):

- There is a role for two different sugar/electrolyte solutions in the treatment of diarrhea in the United States. The solutions would differ in sodium concentration. A solution for deficit replacement should contain 61 to 90 mMol/l of Na^+. A solution for maintenance should contain 40 to 60 mMol/l of Na^+.
- The solution for deficit replacement should be used preferably in a fixed health care facility (physician's office, emergency room, etc.) in overtly dehydrated children.
- The higher sodium solution also may be used in prevention of dehydration or maintenance of hydration but must be accompanied by provision of ample additional low-solute fluids to provide free water.
- A preferred maintenance solution is the lower (40 to 60 mMol/l) sodium solution.
- All the rehydration solutions should be considered "medical foods." (Distribution of medical foods does not require a prescription, but such foods are accompanied by explicit instructions.)

The Economy of ORT

ORT makes the treatment of diarrheal disease more economical in two ways: 1) by diminishing the cost of the rehydration fluids, and 2) by shortening the hospital stay of the pediatric patient admitted for the treatment of dehydration.

The costs of treatment for two hypothetical patients treated by ORT versus intravenous rehydration are compared below.

The first patient is a 10 kg American infant admitted to a large Eastern municipal hospital with 10 percent dehydration. In typical pediatric care in the 1970s, this infant would have a hospital stay of approximately 3 days and would receive intravenous fluids. Typically, he or she would be given nothing by mouth for the first 24 hours and then would have slow introduction of clear fluids and dilute formula over the subsequent 2 days. Some of the costs of treating this infant intravenously would include: sterile intravenous fluids at $1.50/liter (approximately 3 liters might be used in this patient over 2 days); sterile plastic intravenous tubing ($2.62); and at least one sterile butterfly needle ($0.40). The relevant cost of intravenous therapy for the infant would be $4.50 for intravenous fluids, plus $3.02 for sterile tubing and needle and $600 for 3 days of hospitalization.

In the 1980s, this same infant could be treated with ORT and early reintroduction of feeding with a probable hospital stay of only 24 hours. The cost of ORT would be $0.25 per liter for WHO oral rehydration solution. The infant might require 3 liters ($0.75) of the solution, but no sterile tubing or needles would be needed. So the relevant cost of ORT for the infant would be $0.75 for oral rehydration solution, plus $200 for 1 day of hospitalization.

The second hypothetical patient is a 16-year-old, 70 kg east African male who presents with severe cholera during a cholera outbreak. He arrives at the hospital in a severely dehydrated state and in shock. Treated with intravenous fluids, this patient might typically require 20 liters for treatment. This would cost $30 in fluids alone ($1.50 per liter) plus at least $2.00 for sterile tubing and needles. Thus, the total cost for intravenous therapy for this patient would be $32.00.

With ORT, this patient's intravenous fluid requirement could be reduced to 3 liters ($4.50). The patient would also require approximately 26 liters of oral rehydration solution (at $0.15 per liter in east Africa). So the total cost of treating this patient with ORT would be $3.90 for 26 liters of oral glucose/electrolyte solution, $6.50 for 3 liters of intravenous solution, infusion tubing, and a sterile needle to treat shock; the relevant costs total $10.40.

The comparative costs for purely intravenous treatment of a patient with severe cholera in shock, $32.00, versus the use of ORT, $10.40, are notable. Many patients with somewhat less severe cholera can be treated entirely with ORT alone, further reducing the costs. Savings of this magnitude in therapy of diarrheal diseases are of critical importance to less developed countries where financial resources are severely limited in comparison with health care needs.

Conclusion

Diarrheal disease is one of the most important causes of morbidity and mortality in infants and young children in the less developed world. Dehydration is the most common precipitating cause of death in infant diarrhea, and malnutrition is the most important chronic consequence of such diarrhea.

Even during diarrheal infection, the intestine maintains its ability to absorb glucose and many amino acids; as glucose is absorbed, sodium and water also are absorbed. This observation has allowed the development of ORT—a highly efficacious form of therapy for diarrheal dehydration that is technologically appropriate for use in less developed countries.

ORT, which involves oral rehydration with solutions containing glucose or sucrose and electrolytes, now is in use worldwide, in programs sponsored by international agencies and national governments. Although the basic breakthrough has been made, efforts to improve the solutions and to increase the accessibility to ORT by those in greatest need are continuing.

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Case Study B.—The Development of a Malaria Vaccine

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Introduction

The recognition that malaria stimulates natural immunity gave rise to the hope that a protective immune response could be reproduced artificially. Experiments in animals and humans have shown that this is indeed feasible. Malaria vaccine research today is directed at identifying the immunity-stimulating portions of the parasite or its products, producing them in quantity, and introducing them into the human body in such a way that they stimulate immunity without causing disease.

The undertaking is an ambitious one. A parasite vaccine is difficult to engineer simply because a parasite is so much larger and more complicated than a virus or bacterium (targets of all familiar vaccines), and carries a multiplicity of antigens. The problem is complicated by the fact that antigens on the malaria parasite vary according to the species of *Plasmodium* involved and the stage of the parasite's development. Vaccines are now being developed against the various types of malaria in all stages of the parasite's life cycle. The ultimate vaccine will probably combine antigens to various stages. The main target species is *P. falciparum* because it is so often lethal and the stakes in preventing it are highest, though eventually a vaccine might protect against several species.

Although natural immunity to malaria develops slowly, over a long period of time, and requires repeated contacts with the parasite, vaccine researchers are encouraged by the prospect that an artificial vaccine may be able to improve upon nature. By using only the immunity-producing antigens—and not the many proteins and contaminants carried by a whole parasite, or in less pure vaccine preparations—they expect to be able to sidestep the multiplicity of immune reactions that are triggered by the intact parasite, many of which may actually favor the parasite's survival.

Each of the three main life stages of the malaria parasite—the sporozoite, the merozoite, and the gamete—has now been shown, under certain conditions, to produce immunity in birds, rodents, and monkeys. Irradiated sporozoites, in an ingenious experimental system (mosquitoes do the inoculating) have also succeeded in immunizing a few human volunteers.

The farthest advanced work centers on the sporozoite, the infectious form transmitted to people by mosquito bite. The immunogenic sporozoite antigen has been isolated and characterized for several parasite species. The gene that codes for the sporozoite antigen of a monkey malaria has been identified, and the antigen reproduced through genetic engineering. Moreover, because this antigen has proved to have a relatively simple structure, it has been possible to synthesize it, and the synthetic antigen has been used to immunize rodents and monkeys. A dramatic announcement in the summer of 1984 revealed success in cloning and elucidating the structure of the antigenic sporozoite protein of the first human malaria, *P. falciparum*.

Although sporozoite research has captured the lead, most malaria vaccine programs are concentrating on other forms of the parasite. Progress has been impeded by the fact that blood stage parasites carry many antigens, most of which do not elicit a protective immune response. Moreover, blood stage parasite antigens vary not only between stages and between species, but also from one geographic strain to another. Once the antigens are identified, the strategy for producing a vaccine is similar to the strategy for sporozoites. One set of blood stage antigens from a human malaria has been cloned, and these antigens are being studied to see if some of them are candidates for a vaccine.

A vaccine against gametes, sexual forms that occur in the mosquito, would not prevent disease symptoms but would block disease spread. Gamete antigens that can elicit antibodies capable of preventing parasite fertilization have recently been identified and are under study.

Several government and international organizations, whose support has financed vaccine research, have already met to make preliminary plans for field trials of a sporozoite preparation; such trials are expected to get under way by 1986 or 1987, although the details have not yet been worked out.

The eventual large-scale production of a malaria vaccine or vaccines, complicated by patent issues, and the difficulties of delivering a vaccine to large numbers of people, many of whom inhabit remote and impoverished areas, will require thoughtful attention and cooperation from the international community.

A first-generation sporozoite malaria vaccine is on the threshold of becoming a reality. Scientists are convinced that they have at their fingertips the capability of identifying and producing those elements of the malaria parasite that evoke an immune response, and public health planners anticipate the start of clinical trials in 1986 or 1987.

This is not to say that work is complete. This first "vaccine," even if it proves safe and effective, would probably constitute no more than one component of the ultimate vaccine. Many formidable hurdles—biological, immunologic, and chemical—are yet to be met, and the logistical problems of field testing, mass producing, and delivering a vaccine to target populations are enormous. Nevertheless, the prevailing mood is one of great optimism.

The development of a malaria vaccine has been propelled by two distinct currents. One is the failure of an international effort to eradicate malaria, due in large part to the emergence of mosquitoes resistant to pesticides and malaria parasites resistant to antimalarial drugs. The other is the recent explosion in biotechnology, which has provided scientists with tools that have swept away obstacles that seemed insurmountable less than a decade ago.

A program to rid the world of malaria, which at the time was estimated to affect 250 million persons annually, with 2.5 million deaths each year (5), was launched by the World Health Organization (WHO) in 1957. Through a combination of large-scale spraying of insecticides and medical treatment, the program succeeded in eliminating malaria or greatly reducing it in about 80 percent of the target areas by the mid-1960s. Just a few years later, however, the picture had begun to change dramatically. In Sri Lanka, where the number of cases had shrunk from 3 million to only 18 reported cases, an epidemic of malaria broke out, and more than 1 million people were affected (128). Although malaria remained vanquished in temperate regions of the world, the disease was making a strong comeback in many tropical areas.

To some extent, the resurgence reflected the difficulties of carrying out such an ambitious and complex program in countries that lack a strong, central, public health program (63,95,96). More fundamental, however, was the fact that more and more malaria-carrying *Anopheles* mosquitoes were becoming resistant to DDT (dichloro-diphenyl-trichloroethane) and other insecticides. By 1981, insecticide resistance had appeared in 51 species of *Anopheles* mosquitoes (96). Because alternative insecticides, most of them petroleum products, are often too expensive for Third World countries to use, spraying efforts have been curtailed (128).

The first reports that the malaria parasite could resist the effects of chloroquine appeared almost simultaneously in several countries of Southeast Asia and South America around 1960. Resistant strains of *P. falciparum*, the species that causes the most severe disease, have now surfaced in more than two dozen countries in Latin America, Asia, and Africa (128). New drugs are being developed, but early reports indicate that the parasite can become resistant to them, too.

In short, despite extensive research efforts, there is little affordable on the shelf to fend off a disease for which one-third of the world's population is at risk, which strikes an estimated 150 million persons a year, and which causes at least 1 million deaths each year.

While efforts to conquer malaria through drugs and insecticides have been faltering, research into the immunology of malaria, with a view to developing a vaccine that could prevent its symptoms and its spread, has been surging ahead. One major breakthrough came in 1976, when it first became possible to grow *P. falciparum* in continuous culture, in the laboratory. This ready source of raw material opened the way for a stream of studies on the parasite and its ability to stimulate immunity. The pace accelerated again with the advent of the new biotechnologies. Since 1980, malaria researchers have been using monoclonal antibodies (MAbs) to identify those precise parts of the parasite that produce an immune reaction. They then produce this protective antigen in quantity through recombinant DNA technology, cloning those parasite genes that code for the protective antigens. Alternatively, once the antigen structure has been fully spelled out, scientists can synthesize the antigens chemically.

The Malaria Parasite

Malaria is caused by single-celled protozoan parasites of the genus *Plasmodium*. More than 100 different species are known to cause disease in a variety of animals (24). Four species naturally infect humans: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. Each of the four has a distinctive appearance and life cycle; each produces a somewhat different clinical effect (72). The most dangerous is *P. falciparum*, which can cause severe anemia, kidney failure, and brain damage; it is often fatal, especially among children. In *P. vivax* infection, the typical symptoms—cycles of chills, high fever, and sweating with headache, muscle aches, and nausea—are less severe. Although the disease is not often fatal, relapses can occur periodically for up to 3 years. *P. malariae* infections can persist in the blood, without producing symptoms, for life; chronic infections in children can lead to kidney damage.

Other species of *Plasmodium* cause malaria in a variety of vertebrates—reptiles, birds, rodents, rabbits and monkeys. Several of these have been used as experimental models in vaccine research: *P. gallinaceum* and *P. lophurae* in chickens and ducks; *P. berghei*, *P. yoelli*, *P. vinckei*, and *P. chabaudi* in rats and mice; and *P. knowlesi* in the rhesus monkey. In addition, human malarias can infect certain higher apes and New World monkeys. An important experimental model is *P. falciparum* in the South American owl monkey.

All malaria parasites have a complex life cycle, alternating between vertebrate host and mosquitoes. In vertebrates they reproduce asexually, first in the liver and then, repeatedly, in the red blood cells (erythrocytes). In the mosquito, they reproduce sexually.

Human infection begins with the bite of an infected female *Anopheles* mosquito. As she ingests a blood meal to nourish her eggs, she simultaneously injects a stream of saliva that can contain plasmodial *sporozoites*, which have been clustered in her salivary glands. The motile, threadlike sporozoites quickly leave the bloodstream and lodge in the cells of the liver (*hepatocytes*). Within about an hour, all sporozoites have disappeared from blood circulation.

Over the next week (*P. falciparum* and *P. vivax*) or 2 weeks (*P. malariae*), each sporozoite that has invaded a liver cell becomes a *schizont*, a developmental structure that contains thousands of *merozoites*. Liver stage parasites are known as "exoerythrocytic" forms, to distinguish them from blood stage, or "erythrocytic," parasites. When the schizont is mature, it ruptures out of the infected liver cell and discharges thousands of merozoites into the bloodstream. In *P. vivax* and *P. ovale* malaria, some sporozoites become *hypnozoites*, forms that remain dormant in the liver for months or years before they start to proliferate (62).

Merozoites, released into the bloodstream, invade erythrocytes. The invasion process, which can be observed by microscopy, takes about 20 seconds. *P. vivax* and *P. ovale* parasites invade young erythrocytes, while *P. malariae* preferentially infect mature erythrocytes; *P. falciparum* invades old and young cells alike—one reason why the concentration of parasites in the blood reaches dangerously high levels in *P. falciparum* (82).

Most of the parasites that enter erythrocytes undergo a second round of asexual reproduction, similar to but quicker and less prolific than that in the liver cells. In 2 or 3 days, depending on the species, the intraerythrocytic parasite has developed from young *ring forms* to *trophozoites* to the dividing form, again known as a *schizont*. Red blood cells infected with *P. falciparum* develop "knobs," small, sticky, protrusions of parasite origin that allow the infected erythrocyte to adhere to the lining of small blood vessels while the parasite matures (70,117). The infected cell is thereby prevented from circulating through the spleen, where it could be destroyed.

Depending on the species, each schizont contains 10 to 20 erythrocytic *merozoites*. When the schizont is mature, these merozoites burst out of the erythrocytes and invade yet other red blood cells, thus perpetuating the cycle of infection. It is at this point, when the red blood cells rupture, that clinical symptoms appear; because the cycle can repeat every 48 hours (*P. vivax*, *P. falciparum*, and *P. ovale*) or 72 hours (*P. malariae*), attacks of fever can occur every 2 or 3 days.

Plasmodial parasites thus continue to recycle until they are brought under control through drug therapy or through the host's immune defenses, or until the host dies. If reinfection does not occur, *P. falciparum* infections will generally clear in 1 to 2 years; *P. vivax* and *P. malariae* may last 3 years. *P. malariae*, if untreated, can persist as an asymptomatic infection for decades.

Some of the merozoites that invade red blood cells, instead of developing asexually, differentiate into sexual forms, male and female *gametocytes*. Mature gametocytes, enclosed within the erythrocyte membrane, circulate in the blood, available to feeding *Anopheles* mosquitoes.

Blood ingested by the female *Anopheles* carries the gametocytes into the mosquito's stomach. There, perhaps triggered by changes in temperature and pH, they shed the red blood cell envelope. Male gametocytes rapidly transform into motile, spermlike structures and fertilize the larger, egglike female gametes, forming zygotes.

Forms that develop from zygotes in about a day, called ookinetes, burrow into the mosquito's stomach wall, where they form oocysts; 9 to 14 days later the oocysts rupture and release the motile, threadlike sporozoites. The sporozoites infect the mosquito's salivary gland. One cycle is complete.

The malaria parasite's many life stages present a variety of possibilities for interrupting the infectious cycle by immunization. Potential targets include:

- sporozoites before they enter the liver cells;
- infected liver cells;
- merozoites before they enter the red blood cells;
- red blood cells carrying infectious schizonts; and
- gametes before fertilization occurs.

Each system has its advantages and disadvantages. The ultimate vaccine is likely to combine antigens against several stages of the parasite. A sporozoite component would provide high immunogenicity; a merozoite component would act as a backup, preventing disease should even one sporozoite escape the anti-sporozoite defenses. A gamete component, even though it offers no direct benefit to the individual being vaccinated, would help to prevent disease spread by interrupting the transmission of parasites from the mosquito to a new vertebrate host.

Malaria and Immunity

It is clear from the course of natural infections that the *Plasmodium* parasite, in its various manifestations, can stimulate immunity. Persons who live in areas where malaria is endemic, and who are frequently exposed to infected mosquitoes, gradually develop some immunity. But the development of immunity usually takes repeated infections, over a period of years (82). Vaccines are designed to mimic the natural process, stimulating protection without producing any of the adverse effects that accompany natural infection.

The Immune System

The immune system has evolved to protect an individual from invasion by "foreign" substances, including micro-organisms such as viruses, bacteria, and parasites. Components of the immune system, including white blood cells called lymphocytes, recognize substances as being foreign by features of their chemical makeup that are unlike "self." The chemical entities recognized as such are called "antigens."

Once the immune system recognizes an antigen, it can set in motion a variety of responses designed to rid the body of the invader. One is the production of antibody by a family of white blood cells known as B-lymphocytes, or B-cells. Antibody is a substance that "matches" the invading antigen and can inactivate it or speed its uptake by scavenger cells. Another set of responses involves T-lymphocytes, or T-cells. Some subsets of T-lymphocytes work in collaboration with B-lymphocytes, helping either to induce or suppress the production of antibodies. Other T-lymphocytes produce potent chemicals (one example is interferon) that call into play yet other cells, and other responses. Other types of immune cells, including "macrophages" and "monocytes," are scavengers equipped to take up and digest foreign molecules and micro-organisms; natural killer cells attack tumor cells and perhaps aid in the elimination of parasites.

Some cells of the immune system become "memory" cells. After the host's initial encounter with a specific antigen, the body's defenses are primed to attack it quickly: the individual acquires immunity.

Natural Immunity

P. falciparum typically takes its greatest toll among children. Infants are protected temporarily by virtue of antibodies they receive from their mothers in breast milk (92). But by the second year of life, children who live in highly endemic areas become victims of severe and recurrent attacks. Those who survive gradually acquire immunity; by 5 to 10 years of age they show few or no further symptoms of disease. Immune adults rarely experience acute attacks. However, such immunity is generally not complete. Even though the individual develops no symptoms, small numbers of parasites may continue to cycle through the red blood cells. The combination of clinical immunity and continued low-grade infection, a condition known as "premunition," reflects a balance between parasite survival and host resistance. Importantly, the person with asymptomatic low-grade parasitemia remains a reservoir for transmitting the disease.

If a person, once recovered, is not re-exposed to infection, immunity gradually wanes. Symptoms may also flare up in otherwise immune individuals when the immune system is disturbed by events such as surgery or pregnancy. Pregnant women in endemic areas, especially those pregnant for the first time, are much more likely than their nonpregnant counterparts to contract acute *P. falciparum* malaria.

Innate Resistance

Some people inherit traits that make them naturally resistant to malaria. In general, these involve some peculiarity of the red blood cell that makes it inhospitable to *Plasmodium*. Because they favor human survival where malaria is endemic, these traits have become prevalent in such areas.

One example is sickle-cell hemoglobin, which is common in areas of west Africa where malaria is widespread. Under conditions of low oxygen tension—which prevail in the small blood vessels where parasite-infected red blood cells sequester—parasites within cells containing sickle-cell hemoglobin die (33). Although children with sickle-cell hemoglobin develop *P. falciparum* malaria, the disease is much less likely to be fatal than in children with normal hemoglobin.

Another trait that confers resistance to malaria involves a receptor(s) on the surface of red blood cells; the receptor is a prerequisite for parasite invasion. It has long been known that most west Africans and many American blacks are completely resistant to infection with *P. vivax*. These persons are also known to be "Duffy blood-group negative": their red blood cells lack genetically determined surface markers known as Duffy antigens A and B. Studies with parallel infections using *P. knowlesi*, a monkey infection similar to *P. vivax*, indicate that these Duffy antigens are closely associated with, if not identical to, the specific receptors that merozoites recognize. Cells lacking these receptors are unable to form a junction with the parasite (74).

Malaria Antigens

Malaria parasite antigens are remarkable for their diversity. Each species and stage of the parasite carries its own characteristic surface structures that mark it as immunologically distinct. Antigens of blood stage parasites, but apparently not sporozoites (132), also differ from one strain to another (67).

To offer protection, the immune system must tailor a response to fit each variation. A person who is immune to *P. falciparum*, for instance, can be susceptible to infection with *P. vivax*, and vice versa. Persons immune to *P. falciparum* in one country—or in one part of a country—may become infected with a different strain of falciparum when they travel. Even within a given area, several strains may coexist; what were once thought to be "relapses" may possibly represent a series of infections with different strains. This phenomenon may also explain the slow development of natural immunity, over the course of many infections (19,53).

In addition, some malaria parasites exhibit antigenic variation. In response to changes in their environment (for instance, the host's deployment of effective immune defenses), the parasite changes surface antigens (53,54).

Immune Responses

A malaria infection stimulates a spectrum of immune responses, not all of them beneficial. These include the production of antibody by B-cells and the participation of various sets of T-cells, as well as the activation of a variety of nonspecific responses, including macrophages, monocytes, and natural killer cells.

Antibodies are clearly important to immunity against malaria. Persons living in endemic areas develop increasing levels of serum antibodies to sporozoites as well as to blood stage parasites as they build up immunity (80). However, much of the antibody produced in response to malaria infection is nonspecific. Other antibodies, though specifically matched to certain parasite antigens, are not protective.

Some antibodies, however, are both specific and protective, and their role has been established in several ways. For one thing, immunity can be transferred passively, by taking serum from immune individuals or experimental animals and injecting it into nonimmune individuals. In a dramatic clinical demonstration, serum from immune adults living in west Africa was given to 12 infants with severe malaria; it cured their symptoms and sharply reduced the levels of asexual blood stage parasites, though the protection lasted only a short time (17). However, it did not affect levels of circulating gametocytes—an early indication that the asexual and sexual stages carry different antigens.

It is also possible to induce protective antibodies by vaccinating animals with antigens from defined stages of the malaria parasite. Like immune serum, MAbs to sporozoites, merozoites, and gametocytes can be used to transfer passive immunity (97) and to inhibit the growth of parasites in vitro (129).

Antibody appears to prevent sporozoites and merozoites from entering their target cells; it can cause them to agglutinate; it may coat them so they become attractive targets for macrophages; it can prevent gametes from forming zygotes.

T-cells are essential for the development of immunity in malaria (56,110). In addition to their major role, which is assisting B-cells to produce antibody, they appear to secrete mediators that recruit and activate other immune cells, including macrophages and monocytes. They may possibly also exert a direct toxic effect on parasites (36).

Several types of nonspecific mechanisms (that is, those which do not depend on the recognition of a particular malaria antigen) can affect the malaria parasite. For one, general potentiators of the immune system, which carry no malaria antigens, can trigger general cell-killing activity. Malaria-infected animals also have increased numbers of macrophages in the spleen, liver, and bone marrow. These activated macrophages are probably responsible for the high levels of interferon, a natural immunopotentiator, seen in such animals. The macrophages can also secrete other soluble substances, monokines, that can trigger immune activity (10).

Natural killer cells are another nonspecific immune defense. Although their known main target is tumor cells, they can also attack virus-infected cells. Levels of natural killer cells increase in people recovering from *P. falciparum* malaria. Also, strains of mice that have high levels of natural killer cells are more resistant to plasmodial infection than mice with low levels. When mice are immunized with irradiated sporozoites, levels of both natural killer cells and interferon rise (91).

Adverse Immune Responses

Although the overall effect of the immune system's activity is to curb the parasite's growth and gradually eliminate it, some of the immune responses elicited by plasmodial infections work to the host's detriment. To begin with, malaria leads to a general suppression of the immune system, impairing the host's ability to cope with other, nonmalarial antigens, as well as the malaria infection itself. Children with malaria, as well as animals infected experimentally, may be unable to mount an effective response when they encounter an antigen for the first time. They may, for instance, respond poorly to vaccination against other diseases such as typhus, meningitis, or measles. Children with malaria are also prone to more, and more severe, viral diseases, including measles, respiratory tract infections, and gastroenteritis.

The immune response to malaria has been described as "hyperactive and at the same time highly inefficient" (126). While some aspects are suppressed, others are overactive. Among the excess of antibodies produced in response to malaria infection are autoantibodies directed against a variety of the body's own tissues. Other antibodies may form potentially damaging immune complexes by combining with soluble parasite antigens. (Soluble antigens are generally considered detrimental to the host, a sort of decoy for the parasite.) Immune complexes are believed to initiate the kidney damage that occurs in malaria; the damage is then thought to be sustained by autoantibodies.

A Malaria Vaccine

Early Vaccine Studies

The earliest reported attempt to vaccinate against malaria dates back to 1910. Two Algerian brothers, Etienne and Edmond Sergent, during the course of "a lifetime of experimental work on malaria control by every means" (41), inoculated birds with killed sporozoites (103).

These experiments came just three decades after Alphonse Laveran, looking through his microscope at blood smears from malaria patients, discovered "elements that seemed to me to be parasites" and identified them as the principal cause of malaria. Only in 1898 did Ronald Ross put an end to theories incriminating bad air (the Italians called it *mal' - aria*) (41), decay, or filth, by demonstrating that the source of malarial infection was the mosquito.

Vaccination was not attempted again for several decades. By that time a variety of studies had established that antiparasite antibodies existed, could be detected, and could be used to transfer immunity passively in monkeys (14).

During the 1930s and 1940s, it became clear that vaccination was feasible. Birds, rodents, and eventually primates were immunized against malaria, using one of two relatively accessible forms of the parasite, either sporozoites inoculated by feeding mosquitoes, or the blood stages contained within infected erythrocytes. The usual strategy was to inactivate or kill the parasites, inject them into an experimental animal, wait for immunity to develop, and then challenge the animals by exposing them to infection with virulent parasites.

The first successful vaccine used *P. gallinaceum* sporozoites to immunize fowls. Fifty percent of the birds were able to survive a subsequent challenge (79).

In a series of experiments in the 1940s, Freund and his colleagues studied killed blood stage parasites. They used mature schizonts, derived from red blood cells and inactivated by formalin, with and without adjuvants, to immunize ducks (114). With adjuvants, they succeeded in protecting rhesus monkeys from an invariably fatal infection with *P. knowlesi* (32).

In 1946, Heidelberger attempted to vaccinate human beings. Using formalin-killed blood stage *P. vivax* parasites, he inoculated a series of patients and volunteers. The effort was not successful (44). According to current opinion, "perhaps the most remarkable result of this trial was that eight injections of antigen given subcutaneously, intracutaneously, and intravenously over a period of 2 weeks did not cause marked reactions in the volunteers" (25).

During the 1950s, while dreams of eradicating malaria through vector control and drug therapy flourished, research on malaria vaccines was relatively quiescent. Lacking any ready source of malaria parasites, and thus malaria antigens, researchers focused on transferring immunity passively, by means of antibody-containing serum from immune individuals, or to newborn animals through maternal milk. These studies led, in the early 1960s, to the demonstration that passive immunity could be effective in humans—not preventing malaria infection but sharply reducing the severity of the disease (17).

The pace did not pick up until the latter half of the 1960s. By that time, the success of the WHO malaria eradication program was in doubt, and several laboratories began to make some headway in malaria immunology.

The U.S. Agency for International Development (AID) began funding research toward a vaccine, an effort that has been sustained and has been at least partially responsible for much progress in this area. The first all-encompassing AID contract—to grow both sporozoite and blood forms in culture, and to isolate their protective antigens—was awarded to researchers at the University of Illinois in 1966; by 1972, the program had expanded into a seven-site network and was still growing (29).

Vaccination With Sporozoites

Most of the immune response to a natural infection with malaria is elicited by blood stage parasite antigens. Sporozoites, however, are also highly immunogenic. Despite the facts that only a relatively small number of sporozoites are inoculated by a mosquito bite and that they spend a very brief time in the host's bloodstream, sporozoites trigger antibody production (81). Furthermore, experimental sporozoite vaccination—without the use of adjuvants—completely blocks infection, but only for a few months.

For a sporozoite vaccine to be effective, it must kill *all* sporozoites. If a single sporozoite escapes, it can infect a liver cell and eventually give rise to up to 40,000 merozoites (in *P. falciparum*) that can then infect red blood cells, creating a fullblown attack of malaria.

A drawback to sporozoite research has been supply. Sporozoites live in the salivary gland of an infected female mosquito. To produce sporozoites, mosquitoes must be raised, then infected by feeding them gametocytes. After the appropriate interval, laboratory workers must dissect the mosquitoes' salivary glands. Even though a single salivary gland teems with sporozoites, dissection is a painstaking, labor-intensive process that is obviously unsuited to large-scale production. Furthermore, the sporozoites must then be purified of mosquito saliva and other contaminants.

The good news is that recombinant DNA technology promises to significantly ease sporozoite research. The protective sporozoite antigen, which is distributed uniformly over the surface of the sporozoite, consists largely of a single antigen and short marker or epitope, repeated several times. This antigen has now been identified, using MAbs, and produced by recombinant DNA technology. Moreover, the repeating epitope of the *P. knowlesi* sporozoite has been synthesized, and used to immunize monkeys (34).

Research in the 1960s and 1970s.—In the late 1960s, Richards revived Mulligan's studies of a quarter century earlier by showing that killed *P. gallinaceum* sporozoites protected birds from challenge with sporozoites, but not blood forms, of the malaria parasite (98). Richards subsequently used the same technique to protect mice against infection with *P. berghei* and *P. chabaudi* (99).

Meanwhile, Nussenzweig and her coworkers at New York University (NYU) had begun what would become a major contribution to the development of a sporozoite vaccine. NYU parasitologists had just succeeded in growing the rodent parasite, *P. berghei*, through the mosquito cycle. Taking advantage of this steady supply of sporozoites, Nussenzweig showed that vaccination was effective. Repeated intravenous injections of X-irradiated *P. berghei* sporozoites protected more than 90 percent of the mice against an otherwise lethal challenge; immunity lasted about 2 months (87).

Subsequent studies elucidated other features of the immune response to vaccination with intact sporozoites:

- Sporozoites are more immunogenic if they are injected intravenously than if they are administered intramuscularly, subcutaneously, or orally (109).
- Not all sporozoites are immunologically equal: only "mature" sporozoites obtained from mosquito salivary glands 17 to 18 days after the mosquito becomes infected are protective; younger sporozoites collected from the mosquito stomach wall are not effective because they lack an immunogenic surface antigen (121).
- Antisporozoite immunity is species and stage specific. Mice immunized with *P. berghei* sporozoites could be infected with erythrocytes (87) or schizonts (123). They were also susceptible to avian and simian malarias (86). However, mice protected with one species of rodent malaria were protected against all other rodent species tested (89).

To get around the problem of injecting material that was contaminated with a relatively large proportion of mosquito salivary gland tissue, the NYU researchers turned to the mosquito for help. Infected female *Anopheles* were exposed to enough X-irradiation to render the parasites they were carrying noninfectious, then they were allowed to bite experimental animals. Mosquito inoculation met with most of the known criteria: mature sporozoites, inactivated by X-irradiation, delivered intravenously, via multiple inoculations. Mice repeatedly bitten by the infected, irradiated mosquitoes developed both circumsporozoite protein antibodies and protection against sporozoite challenge (122).

The technique was soon applied to humans. For 84 days, three volunteers were exposed to a total of 397 mosquitoes that had been infected with *P. falciparum* and then irradiated. On day 98, the volunteers were challenged by exposure to heavily infected but nonirradiated mosquitoes. Two of the three men became infected, but the third resisted infection. The third man subsequently resisted challenge with other strains of *P. falciparum*, but not *P. vivax* (12). It is thought that the two volunteers who were not protected had probably been inoculated with too few sporozoites during the 84-day immunization period.

The same technique was used to immunize another volunteer in 1974 (100). The following year, a volunteer (the researcher himself) was protected sequentially against *P. falciparum* and *P. vivax*; the former immunity lasted 3 months, the latter, 6 months (11).

Early attempts to immunize rhesus monkeys using *P. cynomolgi* were not very successful (18,125). Results were better using multiple injections of large numbers of *P. knowlesi* sporozoites over a period of several months (39).

Recent Sporozoite Vaccine Research.—The search for purified malaria antigens surged ahead when biotechnology made it possible to produce MAbs —that is, antibodies that are secreted from clones of a single, hybrid parent cell and which are thus identical, all equipped to recognize and link to one single, specific antigen. In the case of the malaria sporozoite, MAbs were derived by fusing nonsecreting but long-lived cells from a plasmacytoma (a plasma cell tumor) with antibody-secreting spleen cells from mice that had been immunized with *P. berghei* sporozoites.

The resultant hybridoma secreted a MAb that singled out the immunogenic sporozoite antigen. This antigen, which has a molecular weight of 44,000 daltons (a measure of mass), and is known as "Pb44" (129), is distributed uniformly over the surface of mature sporozoites, but it is not found on the other stages of *P. berghei* (except for the very early stages within the liver). Additional studies identified two more antigens, Pb54 and Pb52 (130), which are recognized by the same MAb; these have proved to be intracellular precursors of Pb44.

The importance of this MAb in immune protection has been demonstrated in several types of laboratory tests (81,97,129). The antibody also prevents sporozoites from invading target liver cells in vitro (51). In addition, the passive transfer of very small amounts (10 μg) completely protects mice against sporozoite challenge. Similar MAbs were produced against *P. falciparum* and *P. vivax* sporozoites (81) as well as against *P. knowlesi* (13).

Work using these MAbs has identified one surface antigen and one intracellular antigen on each of two human malaria sporozoites, Pf58 and Pf67 in *P. falciparum*, and Pv45 and Pv51 in *P. vivax*. One surface antigen, Pk42, and two intracellular precursors, Pk52 and Pk50, have been identified for *P. knowlesi*.

With the availability of genetic engineering, it became only a matter of time until a potentially protective sporozoite antigen was cloned. The first success was reported in 1983 (27). Researchers at NYU extracted messenger RNA from mosquitoes infected with *P. knowlesi*, converted the messenger RNA into complementary DNA, and inserted the complementary DNA into a plasmid. The plasmid containing the genetic material from the *P. knowlesi* was then introduced into an *Escherichia coli* bacterium. Using MAbs to identify the many proteins being produced by the various *E. coli* colonies, they isolated three clones that produced the sporozoite surface antigen.

Analysis of the DNA sequences in these clones showed that they code for an epitope of 12 amino acids, which is repeated 12 times in tandem (35). Because the shortest of the three DNA sequences cloned contains nothing but the repeating unit, it was possible to deduce the amino acid sequence that the DNA codes for. Then they corroborated their deduction by chemically synthesizing it, and showing that it and the native *P. knowlesi* circumsporozoite protein behave identically (35). This peptide was then used to immunize rabbits and monkeys (34).

The announcement that the circumsporozoite protein gene of *P. falciparum* had been cloned, inevitable but still a cause for excitement, came in August 1984 (21). As predicted, a repeating sequence of nucleotides makes up a large portion of the molecule. The surprise is that the repeat is shorter than the corresponding section in *P. knowlesi*, consisting of only four amino acids, with some slight variation. The simplicity of this antigenic protein should be an advantage in the vaccine development work that now is proceeding.

Other avenues of research are yielding ingenious approaches to vaccine delivery and testing. In a twist of genetic engineering, the gene encoding for the circumsporozoite protein of *P. knowlesi* has been incorporated into the genetic material of the vaccinia virus, the agent of smallpox vaccine. When this recombinant vaccinia virus is introduced into a cell, the infected cell produces not only protective vaccinia proteins but also the protective circumsporozoite protein.

The recombinant vaccinia virus system has already been used to vaccinate animals against hepatitis, influenza, and herpes (108). Now rabbits have been vaccinated with a recombinant *Plasmodium*-vaccinia virus, and have responded by developing antisporozoite antibodies (107). Because the vaccinia virus has a large capacity for foreign DNA, it might eventually be possible to incorporate genetic material for antigens to several of the malaria parasite's life stages (107).

A test to measure a sporozoite vaccine's effectiveness—a pivotal concern of field testing—has evolved from studies of the parasite's cycle within the liver. The new test, called the inhibition of sporozoite invasion assay, measures the ability of *P. falciparum* and *P. vivax* sporozoites to invade cultured human hepatocytes. Such invasion is blocked not only by monoclonal antibodies to the circumsporozoite protein, but also by serum from immunized volunteers and serum from persons living in endemic areas. Once the test has been adapted for use in the field, it should be possible to evaluate levels of preexisting immunity in a broad population, and to monitor the effects of a sporozoite vaccine in clinical trials (51).

Vaccination With Blood Stage Parasites

As the form that induces disease as well as natural immunity, blood stage parasites—either mature schizonts in red blood cells or free merozoites—are good candidates for a vaccine, and blood stage antigens are the object of most of the malaria vaccine research in the world today. Although a blood stage vaccine would not block either sporozoite or liver stage infection, those stages produce no symptoms. An effective blood stage vaccine would prevent disease and interrupt further transmission.

Vaccines to blood stage parasites have succeeded in producing immunity in several animal models, including *P. knowlesi* and *P. fragile* in the rhesus monkey and the human malaria *P. falciparum* in the *Aotus* monkey. However, the degree of protection depends on the nature of the antigen used and the way it is administered. Moreover, it is typically necessary to combine the parasite component with an adjuvant, which independently and nonspecifically boosts the immune response; most adjuvants used in animals are unsuitable for use in humans because of adverse side effects.

Another problem is that blood stage parasites are antigenically very complex. In contrast to the sporozoite, which has a single immunodominant antigen with a short repeating epitope, blood stage parasites carry a mosaic of antigens, many of which elicit responses that are not protective. These antigens may vary not only between stages and between species, but also from strain to strain and even in the course of one infection.

Evaluating the effectiveness of blood stage vaccines is complicated by the use of experimental systems that yield many different patterns of clinical immunity. The same preparation—for instance, blood parasites attenuated by irradiation—will protect the rat, but give less protection against more virulent infections in mice or monkeys (15). Additionally, different programs have used various doses and immunizing schedules, and different ways of measuring the outcome.

Malaria vaccine research took a tremendous leap forward, when, in 1976, it became possible to grow *P. falciparum* blood stage parasites in continuous culture in the laboratory. Nevertheless, with current techniques in vitro cultivation is not suitable for large-scale production (24), nor has it eliminated problems of contamination by cells in which the parasites are grown.

Again, biotechnology holds out the promise of solutions. The major challenge now is to identify the protective antigens and produce them through cloning, or possibly, chemical synthesis. Hybridomas are being used to produce MAbs, which are, in turn, being used to isolate antigens that are protective.

Research in the 1960s and 1970s.—Attempts to induce immunity against the erythrocytic forms of malaria parasites have used a variety of approaches. In some cases the animal (mouse, rhesus monkey, *Aotus* monkey) was exposed to a severe or lethal infection (transmitted by parasitized blood), then treated with drugs or dietary manipulation; once cured, the animal was immune. Monkeys given subcurative drug therapy, however, develop chronic recrudescent infections, and each recrudescence is associated with an antigenically different population of parasites (3). Such antigenic variation may contribute to chronic infections and explain the slow development of natural immunity over the course of repeated infections (19,53).

In other immunization experiments, animals were inoculated with parasitized erythrocytes that had in some way been altered: attenuated (weakened by growth in culture), heat-inactivated, killed, or combined with other adjuvants. Sometimes parasite fractions have been used. Such immunization can convert lethal infections to chronic disease, with most animals eventually recovering (*P. berghei* in mice and *P. falciparum* in the *Aotus* monkey), or at least prevent a portion of the animals from dying (*P. knowlesi* in rhesus monkey) (4).

Many blood stage immunization studies have concentrated on the free-living merozoite. Immunity to malaria is at least partly mediated by antibody, and in vitro studies have shown that merozoites are the target of this protective antibody; it prevents their entry into red blood cells (16).

Merozoites have proved to be an effective form of vaccination. When *P. knowlesi* merozoites, combined with Freund's complete adjuvant were used to vaccinate rhesus monkeys, 100 percent of the animals survived this usually fatal infection (77,78). Merozoite vaccines, with and without adjuvants, have also succeeded in immunizing *Aotus* monkeys against *P. falciparum* infection, with 100 percent survival (78, 104). Merozoite vaccines have shown varying degrees of effectiveness in birds, chickens, and rodents.

In Vitro Cultivation.—Until the mid-1970s, merozoites for vaccine studies had to be separated out of suspensions of schizont-infected red blood cells. Scientists had, for more than 60 years, been attempting to develop a steady supply of blood stage parasites by growing them in continuous culture. In 1912, two malariologists, having cultivated the parasite through three cycles (6 days), predicted that continuous culture would be achieved within the year (71).

The approach that finally succeeded in 1976, capping more than 30 years of research by Rockefeller University parasitologist William Trager. Trager, working with James Jensen, devised an apparently simple system that resembles the normal physiological environment. *P. falciparum* blood stage parasites are grown in human red blood cells in a culture medium that is supplemented with normal human serum, in an atmosphere reduced in oxygen and enriched with carbon dioxide. As schizonts mature and rupture, extracellular merozoites accumulate in the culture medium (115). A similar system was devised independently by scientists at the Walter Reed Army Research Institute, though the parasites recycled for only 21 days (43).

Because the system is easy to reproduce, it was soon in widespread use and was improved upon. In addition to providing material for immunization experiments, the in vitro culture technique proved useful for screening antimalarial drugs, for studying parasite-red blood cell interactions, and for exploring the cellular abnormalities underlying sickle cell anemia (116).

It also benefited research on vaccines against other stages of *P. falciparum*, because with further manipulation, asexual blood forms could undergo development into gametocytes. These are proving useful not only for gamete vaccine research, but also—when fed to mosquitoes—as a means of producing sporozoites.

Recent Blood Stage Vaccine Research.—The search for protective blood stage antigens—on the surface of either the merozoite or the infected erythrocyte—has been hindered by their tremendous complexity: *P. falciparum* appears to carry about 40 antigens (47,93). Blood stage antigens also change as the parasite develops and matures.

Monoclonal Antibodies.—MAbs made with hybridoma technology have been raised against the blood stages of several species of malaria—*P. yoelli* (81), *P. knowlesi* (23,28), *P. falciparum* (94), and *P. berghei* (93,94)—but not against parasite antigens on the erythrocyte membrane (73).

Because most MAbs are produced by immunizing mice with the entire schizont-infected erythrocyte, or through mosquito-borne infection, and not by immunization with a pure parasite antigen, the clutch of MAbs that results needs to be sorted out by laboratory screening. Then each MAb is tested for effectiveness: When mixed with merozoites in vitro, will it cause the parasites to agglutinate, or otherwise prevent them from invading red blood cells? When injected into test animals, will it confer passive immunity? If a MAb passes these tests, its corresponding antigen becomes a target for vaccine studies.

Purified Blood Stage Antigens.—Both immune serum and MAbs have been used to identify potentially protective blood stage antigens. In general, such antigens have proved to have relatively high molecular weights, to be synthesized at a late stage in the schizont cycle, and to be processed into smaller, discrete fragments (46).

One type of antibody target is antigens on the surface of the merozoite. Antibody to these antigens prevents merozoites from invading red blood cells by agglutinating the merozoites as they rupture from the infected erythrocyte and also, perhaps, by blocking specific receptors on the merozoites that allow them to recognize and form a junction with the erythrocyte.

One merozoite surface antigen of the rodent malaria parasite, *P. yoelli*, which has a molecular weight of 230,000 daltons, has been used to immunize mice; (47), and a related MAb inhibited *P. yoelli* proliferation in vivo (66). Comparable merozoite surface antigens have been identified in *P. knowlesi* and *P. falciparum* (22,28).

Numerous studies, using human serum and/or MAbs, are examing the structure of these antigens and their role in the immune response (2,93).

Second possible point of attack are antigens on the surface of the erythrocyte. Antibody directed against these antigens could destroy intraerythrocytic parasites in a number of ways. By coating the infected erythrocyte, for example, antibody may make it attractive to macrophages. Alternatively, antibody might imperil parasite survival by locking onto "knobs," thus preventing the infected erythrocyte from sequestering in small blood vessels.

One erythrocyte-associated antigen is known as the S-antigen. Although it stimulates antibody formation, the S-antigen varies from one strain of *P. falciparum* to another; many distinct types of S-antigen occur even within a restricted geographical area. The S-antigen is thus more likely to serve as a mechanism to help the parasite evade host immune defenses than to help the host destroy the parasite (19).

Evidence for another type of erythrocyte membrane antigen comes from studies that show that antibody (or other factors) can damage the *P. falciparum* parasite within infected erythrocytes, causing schizonts to degenerate (94), or inhibiting intracellular growth (57,111). Thus, there may be two (or more) groups of antigens expressed on the parasitized erythrocyte surface which elicit antibodies that can block normal parasite metabolism. A purified erythrocyte membrane antigen from *P. knowlesi* infected cells has been successfully used to vaccinate rhesus monkeys (101).

Genetic Engineering.—In a novel attempt to study blood stage proteins, Australian investigators manipulated the usual gene-cloning procedure. They extracted messenger RNA from the various blood stage forms of *P. falciparum*, copied all of it into complementary DNA, and inserted the complementary DNA into *E. coli*. Then, instead of using a MAb to isolate a single piece of DNA that codes for a particular protein, they used human immune serum containing many anti-*P. falciparum* antibodies to identify the many clones manufacturing immunogenic proteins. The result is a library of several hundred clones that express a wide range of so-called "monoclonal antigens," each of which can be studied in detail. In their ensemble, these clones probably represent a large portion of the antigens carried by *P. falciparum* (59). However, it is not yet clear just what antigens they include. The first of these clones to be examined proved to code for a protein with a molecular weight of 220,000 daltons. Although it contains repeated epitopes, like the sporozoite surface protein, it is an S-antigen that varies from strain to strain (19).

Several laboratories are working to streamline recombinant DNA production of malaria antigens. In one approach, developed at the National Institutes of Health (NIH), the genomic DNA itself (as distinct from copies generated through messenger RNA and complementary DNA) is cut into fragments. These gene-carrying fragments are cloned, and the clones screened with MAbs to find which ones are producing the desired antigens (68).

Vaccination With Gametes

Like sporozoites and merozoites, male and female gametes carry antigens that are capable of provoking an immune response. Gamete immunization has prevented parasite fertilization in chicken, mice, and monkey malarias, and blocked subsequent transmission of the disease.

The strategy for gamete vaccination follows a circuitous route. An individual is inoculated with gamete antigens and makes antibodies to them. When a female mosquito feeds on this person, she ingests not only gametocytes, but also antibodies. In the stomach of the mosquito, after the gametes emerge from the erythrocyte casings, they are exposed to these antibodies, which quickly immobilize the male gametes and prevent fertilization.

Studies have shown that chickens immunized against *P. gallinaceum* gametes produce antibodies that block infectivity in mosquitoes (7,37). Although the chickens were still susceptible to malaria infections, the mosquitoes that fed on those chickens developed no or few oocysts. Subsequently, gamete vaccination against *P. yoelii* infection in mice (69), as well as *P. knowlesi* in the rhesus monkey, totally suppressed gamete infectivity in mosquitoes.

Gamete vaccine research moved ahead when, in 1981, it became possible to culture *P. falciparum* gametocytes with regularity. Previously, blood stage forms would only sometimes develop into gametocytes in culture. When hypoxanthine, a substance found in many body tissues, was added to the culture, gametocytes were found to predictably develop into mature infectious parasites (55).

Two sets of target antigens have been identified on *P. falciparum* gametes. The first is a set of three proteins with molecular weights of 250,000, 60,000, and 55,000 daltons. These antigens occur on both male and female gametes, as well as newly formed zygotes, and are shed shortly after fertilization. Antibodies to these antigens block gamete fertilization and, in the presence of complement, destroy both gametes and zygotes. Unfortunately, these antigens, like some merozoite antigens, may vary within a species (8).

The second target of antigamete antibodies is a single 26,000-dalton protein which is synthesized by the zygote and expressed on the zygote surface. Antibody to this antigen prevents zygotes from developing (8).

The gamete vaccine is known as an "altruistic" vaccine because it does not prevent infection or cure disease, or otherwise directly benefit the person being vaccinated. Rather, it benefits the community by drying up the supply of infected parasites, preventing further malaria spread. As a result, it probably will be used only if it is combined with a sporozoite and/or merozoite vaccine. Moreover, its value in the field may be difficult to prove. The effectiveness of a gamete, or transmission-blocking, vaccine would depend on how long immunity lasts, the proportion of the population that is immunized, and the intensity of transmission. In some parts of Africa, the rate of transmission is so high that a single infected individual can lead to the infection of more than 500 others; in such an area a gamete vaccine would hardly make a difference. In other areas, such as India or Sri Lanka, where the transmission is less intense, such a vaccine, possibly combined with other control measures, would have a chance of eliminating malaria (73).

Vaccination With Liver Stage Parasites

Any possibility of developing a liver stage vaccine was impeded until recently by researchers' inability to grow intrahepatic parasites in culture. Although the liver or exoerythrocytic stages of avian malarial parasites had been grown in continuous culture since 1966, it was not until 1981 that mammalian malarial parasites were induced to grow and develop through a complete cycle, beginning with the entry of a sporozoite into the target cell, through the parasite's development into a liver schizont, complete with the release of merozoites (49). Subsequently, the parasitic infection was carried full cycle. The liver merozoites, injected into mice, caused red blood cell infection, and some of the blood stage parasites in the infected mice developed into gametocytes. Mosquitoes allowed to feed on these mice developed sporozoites, which, when inoculated into the cell culture system, invaded the target cells and became liver schizonts.

The initial work was performed using *P. berghei* in cultured human embryonic lung cells. More recently, scientists have managed to grow both *P. falciparum* and *P. vivax* (as well as *P. berghei*) in a cultured cell line derived from a human liver cancer (48). This advance is particularly significant for *P. vivax* research, since it has not yet been possible to grow *P. vivax* blood stage parasites in continuous cultures.

Using the *P. berghei* system, it is possible to watch as sporozoites encounter target cells, enter them, and develop into trophozoites. The process of attachment and entry, which seems to parallel in many ways the invasion of merozoites into red blood cells, appears to be effected by the circumsporozoite protein (Pb44); conversely, MAbs to the circumsporozoite protein will block sporozoite entry into liver cells (50).

The hepatoma culture system is also being used to discover how some *P. vivax* liver stage parasites, instead of promptly developing into schizonts, lay dormant for long periods of time. When these dormant forms, hypnozoites, later reactivate and develop into schizonts that release merozoites, they produce a relapse. Electron microscopy is being used to document the differentiation of liver parasites into hypnozoites during the first few hours of development (48).

Scientists are currently working to isolate, purify, and characterize the cell receptor that permits sporozoites to enter the liver cell. Once the nature of this receptor is better understood, it may be possible to prevent infection by suppressing these receptors with drugs. Alternatively, if parasite antigens can be detected on the surface of infected liver cells, it may be possible to attack infected cells by linking antimalarial drugs to antibodies that will recognize and join up with these antigens.

The culture of liver stage plasmodia has yielded two major spinoffs. One, the inhibition of sporozoite invasion assay described above, can be used to evaluate the effectiveness of a sporozoite vaccine in the field. The other is the adaptation of the liver parasite-hepatoma culture system to test new antimalarial drugs. As a fast and inexpensive alternative to testing candidate antimalarials in costly and scarce primates, the tissue culture system promises to revolutionize drug development. Both the U.S. Department of Defense (DOD) and WHO are exploring its use.

Funding Sources

U.S. Government, international, and philanthropic institutions spent about $20 million in 1984 for malaria vaccine research. The biggest contributors are AID and the National Institute of Allergy and Infectious Diseases (NIAID) of NIH. The next biggest are the U.N. Development Program/World Bank/WHO Special Program for Research and Training in Tropical Disease (TDR), DOD, the Centers for Disease Control (CDC), and the Rockefeller Foundation. In addition, a few pharmaceutical companies are conducting malaria vaccine research.

In 1984, AID spent close to $8 million on the development of a malaria vaccine. The $8 million figure represents nearly a doubling of the Agency's original commitment. In late 1983, sensing that the goal was within reach, AID requested an increase in funds that would bring its outlay for fiscal years 1983 to 1985 from $11.9 to $22.7 million.

Since launching the malaria vaccine program in 1966, AID has spent roughly $35 million, and expects the program effort to cost an additional $15 to $25 million before a *P. falciparum* vaccine is ready for general use. By way of comparison, AID contributed more than $1 billion (and other countries, an additional $4 to $5 billion) to the WHO malaria eradication campaign since its inception in the 1950s (29). AID funded 14 projects throughout the United States in 1984.

AID also provides a variety of support services. One contractor, in addition to research, is charged with testing and characterizing all materials injected into test monkeys; another produces and supplies selected strains of *P. falciparum*, as well as MAbs, to network laboratories. Looking to the future, AID has hired experts to help contractors structure their research so that it will answer Food and Drug Administration (FDA) requirements, and other experts to counsel on matters of patent rights (30). AID is currently taking the lead in laying the groundwork for clinical trials.

NIAID sponsors both intramural and extramural research on malaria, with a total annual expenditure of close to $4 million (120). Including basic research on topics such as recombinant DNA technology or cell receptors, which NIAID itself does not usually classify as "vaccine research" (90), NIAID spends roughly $2 million for intramural research related to malaria immunology, either in the Laboratory of Parasitic Diseases or the Laboratory of Microbial Immunity. The Institute awards an additional $2 million in grants (not contracts) to about 20 institutions, primarily universities, throughout the United States.

Over the past 15 years, the focus of NIAID's malaria research has shifted heavily in favor of immunology and vaccines, and funding commitments have shown a steady growth. During the first 4 years of this decade, when the tempo of research was rapidly accelerating, NIAID's outlay doubled. However, it represents only a minute fraction of the NIH budget. In 1981, when the NIH was spending a total of $3.6 billion, NIAID's share was $232 million or 6 percent; of this, $27 million went to tropical disease research, and about one-fifth of that was given to all aspects of malaria.

Even though the payoffs may not always be so immediately visible as those of the closely managed, product-oriented AID program, the type of steady support provided by NIAID assures the continuous growth of a broad expanse of knowledge. Beyond achieving their own breakthroughs, or even beyond satisfying intellectual curiosity, such studies generate a rich resource for more intense development projects.

TDR is sponsored jointly by the U.N. Development Program, the World Bank, and WHO. It is funded by contributions from its cosponsors, as well as from the governments of more than 25 countries and from several businesses and foundations.

From its beginning in 1976 through March 1983, TDR had received more than $117 million in contributions: $15 million from the United States, $22 million from Denmark, and $14 million from Sweden, plus $9 million from the U.N. Development Program, close to $5 million from the World Bank, and $7 million from WHO (118). For the 2 years, 1982 and 1983, TDR had budgeted just over $61 million.

Research on all aspects of malaria (chemotherapy and field applications as well as malaria immunology) accounts for about 30 percent of TDR's research and development budget. In 1981, this amounted to $3.2 million. Of this, $1.36 million went to support 40 projects on malaria immunology and vaccines (see chapter 3). Of these, 24 were in the United States (119). Others were in Great Britain, France, Switzerland, and Australia.

Malaria vaccine research within DOD is focused on preventing disease in American troops stationed abroad. Thus, DOD's malaria vaccine efforts give special emphasis to sporozoite and liver stage vaccines, which have the potential of preventing the initial infection and symptoms. AID or TDR efforts are also interested in blood stage vaccines as a means of curbing symptoms and interrupting the parasite's life cycle in areas where the disease is endemic and transmission is heavy.

Within DOD, the Army is the lead service in malaria research, and its work is headquartered at the Walter Reed Army Institute of Research. Navy activities are carried out by the Naval Medical Research Institute.

Although DOD vaccine studies extend back 15 years, studies began to accelerate around 1980, when the new biotechnology opened the possibility of obtaining antigen in purified form. The Army has collaborated with NIH in the effort to clone and characterize a sporozoite antigen; it is also working toward the chemical synthesis of the sporozoite's antigenic epitopes. The Navy has pioneered work on the liver stages of the parasite.

The Army and Navy malaria vaccine groups, each of which employs about 20 persons, work closely together. Expenditures for each of the groups has been estimated to be close to $1 million a year (1,30,45).

CDC of the U.S. Public Health Service participates in malaria vaccine research at several levels. Its primate resource center contains more than 100 New World monkeys of value for research on *P. falciparum* and *P. vivax*; several aspects of malaria immunology are the subjects of in-house research projects; and CDC serves as a reference center for the many strains of *P. falciparum*. It also serves as a source of materials; as of early 1984, CDC has supplied researchers at NYU with 40 million *P. vivax* sporozoites, and was developing millions more. CDC also runs field units on three continents; these may provide appropriate sites for clinical testing.

CDC's annual expenditure on malaria in recent years has been estimated to be under $1 million (30). CDC allocates approximately $160,000 to $200,000 a year in direct support of malaria vaccine development (6).

The Rockefeller Foundation, through its Great Neglected Diseases of Mankind program, spends just under $500,000 a year to fund several projects that are directed toward, but not restricted to, malaria vaccines. The three main projects, which have been funded for each of the last 8 years, are located at Harvard, Oxford, and the Walter and Eliza Hall Institute in Melbourne. The group at NYU has also received grants-in-aid, and is currently receiving funds for work on synthetic vaccine development (125a).

Four industrial organizations are involved in vaccine research. The Burroughs-Wellcome Co. of Great Britain, which funnels profits into research efforts through the Wellcome Trust, has a long-standing commitment to tropical disease research. Since 1979, Wellcome scientists have been working on a malaria vaccine, primarily the genetic engineering of blood stage preparations. Its current outlay is approximately $500,000 a year. In Australia, the recently launched Australian Biotechnical Corp. is now investing an estimated $1 to $2 million annually on malaria vaccine projects. Roche Pharmaceuticals, a leader in the antimalarial drug field, recently initiated a collaboration with the Swiss company Biogen to develop a malaria vaccine, also through genetic engineering. Support is reported to be in excess of $5 million.

The Future of Malaria Vaccine Research

Scientists have at hand an "antigen preparation" for one stage of the parasite (the sporozoite) for two species of human malaria (*P. falciparum* and *P. vivax*), and they are making plans leading to clinical trials. The path leading from there to the successful control of malaria through vaccination is long and uncharted. The challenges ahead include: developing a polyvalent (multiple component) vaccine, demonstrating that it is safe and effective, conducting large field trials in developing countries, producing the vaccines in quantity, and delivering it to the populations at risk.

Developing a Polyvalent Vaccine

Antigens.—An ideal vaccine would likely combat multiple forms of the parasite—sporozoite, blood stage, and/or gamete, and perhaps liver stage, as well as two or more types of malaria—*P. falciparum* and *P. vivax*, perhaps with *P. malariae* or *P. ovale*. Alternatively, different preparations might be prescribed for different populations. A sporozoite vaccine, for instance, might be appropriate for persons whose exposure is limited, such as tourists, whereas a merozoite vaccine could be given to control disease symptoms in an area where malaria is highly endemic; a gamete preparation could be part of a public health campaign to eliminate the disease.

A protective sporozoite antigen for *P. falciparum* and *P. vivax* should soon be ready for testing, but it is likely to take another 2 years or more to isolate and produce pure antigens from the blood stages or gametes. Work on liver stage antigens is still preliminary.

To counter the problems of antigenic variation, researchers are exploring a variety of possibilities. These include presenting a parasite structure that is not normally antigenic to the host (but which is common to all of the strains of a species) in such way that it becomes antigenic. Alternatively, they might be able to identify parasite surface structures that play such an important role (for instance, those that enable merozoites to attach to or invade red blood cells) that they should be the same in every strain.

Fortunately, the protective antigen from the sporozoite does not appear to vary from strain to strain (132). For a blood stage or gamete antigen to be useful for a vaccine, however, one must be found that is common to all parasites of a given species, or at least have limited variability. Current experimental work indicates that although a large number of unique geographically defined antigens exist, other antigens are common to many strains. Researchers are now working to discover such antigens, and determine how they might be manipulated for immunization purposes.

Adjuvant.—All experimental malaria vaccine preparations except the sporozoite have required the use of an adjuvant. Unfortunately, the adjuvant that has been most successful in animal studies, Freund's complete adjuvant, produces side effects that make it unacceptable for human use.

Several alternatives are being explored. A bacterial derivative and another substance have both successfully replaced Freund's complete adjuvant in immunizing *Aotus* monkeys against *P. falciparum* (105,106); however, their effects in humans are not known. Parasite-specific MAbs have also worked as an adjuvant, enhancing the effects of blood stage vaccination of mice (42).

Antigenic Variation.—Multiple strains of *P. falciparum*, each with unique antigens, have evolved. Different strains are found not only in different parts of the world, but within given geographic areas. Moreover, some parasite populations appear capable of changing antigens over the course of an infection.

Demonstrating Safety and Efficacy

A likely scenario for the trial of a candidate vaccine begins with several months of testing for toxicity and carcinogenicity in mice and rabbits. Next the vaccine would be tested in primates to see that the preparation produces no discernible adverse effects and that it does stimulate a protective immune response. Assuming that all is going well, the investigators will file an Investigational New Drug application with FDA.

The first clinical tests (which might begin as soon as 1985, under ideal conditions) will use healthy male volunteers, recruited either from a university setting, the military, or industry, and including some of the scientists themselves (29). Again, the first round would be to make sure that the preparation produces no adverse effects. A second round, lasting 18 months, would be designed to answer questions of efficacy: Does it stimulate antibody production? Are these antibodies protective? How long does protection last? In the case of an AID-sponsored vaccine, these volunteer studies would then be replicated in endemic areas, using local volunteers and personnel (29).

Conducting Field Trials

The pilot vaccine will first be tested in healthy males, then healthy nonpregnant females, and then in pregnant females, and children. In addition to the basic issues of safety and efficacy, new questions will arise in endemic areas. Will persons whose immune responses have been dampened by previous malaria infections respond in the same way as volunteers who have never had the disease? Will nutrition affect the body's ability to respond? What about antimalarial drugs?

Neither sites for field trials nor strategies have been arranged. AID, which will work closely with WHO in setting up, and to some extent funding, clinical trials, had convened two planning meetings by mid-1984 with representatives from DOD, CDC, FDA, NIH, and the Pan American Health Organization, as well as WHO. In March 1985, clinical trials will be the focus of a meeting of the scientific working group of WHO's Malaria Immunology program.

For the results of the trial to be meaningful, epidemiologists will need to have mapped patterns of malaria transmission in the test area, and documented the extent of preexisting immunity. The trials will need to be carefully planned and closely supervised, conducted by skilled personnel working in close cooperation with the test population. Those people who are vaccinated will have to be closely monitored and treated when necessary.

Producing a Vaccine

The research institutions in which vaccines are being developed are not geared to produce large quantities of vaccine material; that formidable task will be the concern of pharmaceutical or genetic engineering companies.

The ensuing interrelationships among scientists/universities, research sponsors such as AID and WHO, and industry lead to a tangle of conflicting interests. Who "owns" the discovery? Who should make a profit from it? What are the incentives for genetic engineering/pharmaceutical companies to get involved? Who will buy a vaccine—AID, WHO, DOD, philanthropic foundations?

The issues took shape in 1981, following the Supreme Court's ruling that biologicals are patentable. NYU filed patents for the Nussenzweig group's work (presumably involving MAbs used in identifying and cloning the circumsporozoite protein). When NYU entered into negotiations with a genetic engineering firm, Genentech, to produce the circumsporozoite protein, Genentech asked for exclusive license to market the vaccine.

WHO, which had long supported the NYU work, and which represents many developing countries, held fast to its contractual requirements for public access to work that it supports. AID, another Nussenzweig sponsor, holds patent rights in the United States under Federal law; AID asked NYU to submit the requisite "petition for greater rights."

The conflict dissipated in 1983 when NYU and Genentech dropped their plans to work together. Genentech said it was too busy with other projects; NYU developed superior genetic engineering capabilities of its own. The issues raised, however, remain unanswered.

To date, seven patents have reportedly been applied for in the United States by four different laboratories (30). Four involve sporozoite vaccines, and three are related to merozoite vaccines.

In the meantime, AID is in the process of revising the patent language in its contracts. The new U.S. patent law, which took effect in 1981, allows grant recipients to take out patents on Government-sponsored work, providing the Government is allowed royalty-free use of the invention. AID's goal is to make sure that any agreements struck between its contractors and industry will not impede a vaccine's getting to the market. AID would also like the vaccine to be available to Third World countries and to the U.S. military on a cost-plus basis (58).

AID has held discussions with six domestic companies possibly interested in producing a vaccine. At this time it seems likely that vaccine development in this country will proceed under the Orphan Drug program of FDA (30). Overseas companies, particularly those that have established working relationships in the developing countries where malaria is prevalent, have also expressed some interest. Parke-Davis, the sole U.S. drug company to be involved in malaria vaccine development in the past, and which worked through the AID program, pulled out of the field when the company was sold in the late 1970s.

Delivering a Vaccine

In order for a malaria vaccine to alleviate disease and prevent death, it must reach the people who inhabit those parts of the tropics, often impoverished and remote, where the disease is prevalent. To accomplish this, it will be necessary to raise funds, build an excellent logistical support system, and train personnel. The success of such an effort will depend on close collaboration among international organizations, industry, philanthropic foundations, and national health systems.

The history of the human battle against malaria has been marked by a series of overly optimistic expectations followed by disappointments (41), and the hopes for a vaccine may prove no exception. However, the flood of progress has been so strong, and the possibilities created by the new technologies so vast, that researchers are resorting to phrases like "the most incredible time in the history of malariology."

Conclusions

Having watched the most pressing problems of the 1970s—antigen supply and purity—give way before the wonders of genetic engineering and protein chemistry, scientists are confident that, with ingenuity, they will be able to meet today's challenges. Perhaps it will be possible to boost an antigen's immunogenicity by presenting it to the host in a new way, or to prolong immunity by developing slow-releasing antigens, or to "vaccinate" people by incorporating a parasite gene into bacteria that normally inhabit the gastrointestinal tract. Louis Miller of NIAID likens the search for solutions to standing next to a wall: "Suddenly someone puts a hole in it and beyond are vistas we've never imagined" (73).

The problems of testing, production, and delivery are no less imposing, but again the outlook is optimistic, and planners are pressing ahead. Moreover, the liaisons and lessons of the WHO malaria eradication campaign should stand the vaccine effort in good stead.

Just what form the first vaccine will take, and where and when it will appear cannot be predicted, but, according to Miller, "there is no question that vaccines will be developed against malaria; vaccines have been successful in every animal model tested" (73).

The sentiment is a venerable one in malaria research. In 1897, Ronald Ross, in an attempt to prove that the mosquito was the source of human infection, faced the prospect of dissecting thousands of mosquitoes. Undaunted, he wrote: "The things are there and *must* be found. It is simply a matter of hard work" (41).

Not even its most enthusiastic proponents expect a vaccine, of itself, to subdue malaria. To be successful, a vaccine must be complemented by both improved vector control and better drugs—and fresh, creative approaches to both are being explored. These include mosquito-killing bacteria, mosquito-devouring fish, and mosquito-debilitating micro-organisms, on the one hand, and a Chinese herbal remedy, on the other. A three-pronged attack, combining vaccine(s), drugs, and vector control, provides the best chance yet of bettering the lives of millions.

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Appendixes

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Appendix B.—Method for Analysis of Tropical Disease Research Funding

Description of Methodology

Much of the information included in OTA's analysis of tropical disease research funding in chapter 3 was taken from documents made available by the organizations that fund biomedical research in tropical diseases. These documents were supplemented, in many cases, by discussions with relevant officials of the organizations. Some organizations assembled information specifically for OTA's analysis.

Limitations of the Data

One difficulty in developing information about funding for research on tropical diseases is that the researcher can never be sure that every relevant funding source has been identified. The sources of funding described in OTA's analysis may not include every funding source, but do include the great majority of organizations engaged in tropical disease research funding in the United States.

A second difficulty in developing information about tropical disease research funding is that organizations vary in the definitions they use for biomedical research activities. Because of the attention that has been given to the six tropical diseases included in the Special Program for Research and Training in Tropical Diseases (TDR), many organizations identify research efforts in these diseases specifically. In some cases, however, research activities that may be directly relevant to one of these diseases may be allocated to another research type when data are reported.

TDR, for example, reports disease-specific information for malaria, schistosomiasis, trypanosomiasis, leishmaniasis, filariasis, and leprosy, but has additional separate categories for transdisease research. Transdisease research, such as investigations in vector biology and control, may or may not be directly concerned with any one of the six TDR diseases. Thus, without reviewing the research project proposals or protocols for each transdisease investigation, a researcher may not be able to determine how funds are allocated among the six diseases. The figures for TDR in OTA's analysis exclude TDR projects funded under the transdisease categories, except in the sections of the analysis concerned with research objectives and recipients.

The basis of the classification scheme used to collect and store information about biomedical research activities at the National Institute of Allergy and Infectious Diseases (NIAID) is the causative organism. NIAID compiles data on research in the six diseases of TDR for its own reporting purposes, and these data are used in OTA's analysis. However, NIAID research on diarrheal diseases and acute respiratory infections (ARIs) is identifiable only by causative agent, and for the most part lies outside the areas identifiable as tropical disease research.

Information about the U.S. Agency for International Development's (AID) funding program is difficult to break down beyond broad categories of expenditure, because funds are allocated to categories which may include several grants and contracts concerned with similar objectives but with different timetables. Lists of awards made through AID's Small Grants Program are available from the Office of the Science Advisor and the National Research Council's Board on Science and Technology for International Development (BOSTID). However, these lists identify the date of the award, but do not identify the funding level for each award by year. For this reason, OTA used the total amount of grant funds committed in the year in which the grant was awarded.

The information about the U.S. Department of Defense (DOD) in chapter 3 was provided by officials at the U.S. Army Medical Research and Development Command, who were kind enough to compile the data according to the study criteria. The officials reported to OTA that DOD data are not routinely collected by disease type, but rather by managerial funding category. The officials also pointed out that the precise amount of funds for each category may vary as priorities are reassessed and changes made in allocations.

Data about the tropical disease research activities of the Centers for Disease Control (CDC) were assembled for OTA by the Centers' Financial Management Office.

The Rockefeller Foundation and the Edna McConnell Clark Foundation publish a list of awards made each year. By reviewing this list, OTA was able to allocate awards to the appropriate categories of interest.

Pharmaceutical companies do not make data available detailing their commitment to biomedical research. The information in OTA's analysis about pharmaceutical companies, therefore, is not complete and is largely anecdotal.

Appendix C.—Glossary of Acronyms and Terms

Glossary of Acronyms

AID	—U.S. Agency for International Development
AIDS	—acquired immunodeficiency syndrome
BCG	—Bacillus Calmette-Guerin (vaccine)
BOSTID	—Board on Science and Technology for International Development (NAS)
BTI	—*Bacillus thuringiensis israeliensis*
CATT	—Card agglutination test for trypanosomiasis
CDC	—Centers for Disease Control (DHHS)
CDD	—Program for Control of Diarrheal Diseases (WHO)
cDNA	—complementary DNA
CF	—complement fixation (test)
CIE	—counterimmunoelectrophoresis
COA	—coagglutination (test)
COPT	—circumoval precipitin test
CRL	—Cholera Research Laboratory (Pakistan)
DDT	—dichloro-diphenyl-trichloro-ethane
DEC	—diethylcarbamazine
DHF	—dengue hemorrhagic fever
DHHS	—U.S. Department of Health and Human Services
DNA	—deoxyribonucleic acid
DOD	—U.S. Department of Defense
DPT	—diphtheria/pertussis/tetanus (vaccine)
ELISA	—enzyme-linked immunosorbent assay
EPI	—Expanded Program on Immunization (WHO)
FCA	—Freund's complete adjuvant
FDA	—Food and Drug Administration (DHHS)
FIC	—Fogarty International Center for Advanced Study in the Health Sciences (NIH)
GMI	—Gorgas Memorial Institute of Tropical and Preventive Medicine
GML	—Gorgas Memorial Laboratory
GND	—Great Neglected Diseases of Mankind (program) (Rockefeller Foundation)
HI	—hemagglutination inhibition (test)
ICDDR/B	—International Center for Diarrheal Disease Research, Bangladesh
ICMRT	—International Center for Medical Research (Calcutta)
IFA	—indirect fluorescent antibody (test)
IgG	—immunoglobulin G
IgM	—immunoglobulin M
IHA	—indirect hemagglutination assay
ILRAD	—International Laboratory for Research on Animal Diseases
IMMAL	—Scientific Working Group on the Immunology of Malaria (TDR)
IND	—investigational new drug
INH	—isoniazid
IOM	—Institute of Medicine (NAS)
IPM	—integrated pest management
LA	—latex agglutination (test)
LRTI	—lower respiratory tract infection
MAbs	—monoclonal antibodies
mRNA	—messenger RNA
NAMRU	—U.S. Naval Medical Research Unit
NAS	—National Academy of Sciences
NIAID	—National Institute of Allergy and Infectious Diseases (NIH)
NIH	—National Institutes of Health (DHHS)
NRC	—National Research Council
NYU	—New York University
ORT	—oral rehydration therapy
PAHO	—Pan American Health Organization
PAS	—para-aminosalicylic acid
PMA	—Pharmaceutical Manufacturers Association
PPD	—purified protein derivative
P/S	—pyrimethamine/sulfadoxine
PSTC	—Program in Science and Technology Cooperation (AID)
RIA	—radioimmunoassay
RNA	—ribonucleic acid
RPHA	—reverse passive hemagglutination (test)
RSV	—respiratory syncytial virus
RVF	—Rift Valley fever
TDR	—Special Program for Research and Training in Tropical Diseases (U.N. Development Program/World Bank/World Health Organization)
T/S	—trimethoprim/sulfamethoxazole
UNDP	—U.N. Development Program
UNICEF	—U.N. International Children's Emergency Fund
URTI	—upper respiratory tract infection
VSG	—variant surface glycoprotein
WHO	—World Health Organization
WRAIR	—Walter Reed Army Institute of Research

Glossary of Terms

Acquired immunity: Disease resistance in a person or animal acquired after birth. Such immunity may be active or passive.

Acquired immunodeficiency syndrome: See *AIDS*.

Active immunity: Disease resistance in a person or animal due to antibody production or other responses of the immune system after exposure to a disease-causing agent or a vaccine. (Compare *passive immunity*.)

Acute: Having a sudden onset, sharp rise, and short course. (Compare *chronic*.)

Acute respiratory infections (ARIs): A group of infections of the respiratory tract caused by viruses, bacteria, or mycoplasma, or very rarely, by fungi, protozoa, and the secondary effects of worms. Examples are pertussis, influenza, diphtheria, and measles. For clinical purposes, ARIs are divided between the upper and lower respiratory tracts. Upper respiratory tract infections occur around the teeth, gums, sinuses, throat, tonsils, epiglottis, middle ear, larynx, and trachea. Lower respiratory tract infections, in the lungs and bronchi, include various types of pneumonia and bronchitis.

Adenovirus: One of a group of viruses that causes upper respiratory disease and pneumonia.

Adjuvant: A substance added to the main ingredient of a prescription or solution to increase its effectiveness.

Aerosolized vaccine: A vaccine administered through the nose and mouth by inhaling a vapor, rather than through injection or ingestion.

African sleeping sickness: Infection by *Trypanosoma brucei gambiense* (chronic form found in west Africa) or by *T. b. rhodesiense* (acute form found in east Africa). Also called "African trypanosomiasis." (See also *trypanosomiasis*.)

African trypanosomiasis: See *African sleeping sickness*.

Agglutination: The process by which cells or particles adhere and form visible clumps, a process which is exploited in many diagnostic tests.

AIDS (acquired immunodeficiency syndrome): A disorder characterized by an acquired (not inherited) deficiency of the immune system probably caused by a retrovirus known as HTLV-III, which is complicated by a rare type of cancer (Kaposi's sarcoma) or infections caused by micro-organisms that usually do not produce infections in healthy individuals.

Amebiasis: Infection of the colon with amebae.

American trypanosomiasis: See *Chagas' disease*.

Animal reservoir: See *reservoir, host*.

Antibiotic: A chemical substance produced by a micro-organism that is administered to fight infections, usually bacterial infections, in humans or animals. Examples are pencillin, tetracycline, and erythromycin.

Antibody: A serum protein (immunoglobulin) molecule produced by white blood cells in response to exposure to a specific antigen, and characterized by specific reactivity with that antigen. At present, five classes of antibodies are distinguishable. Most of the antibodies that circulate in the blood are immunoglobulin G (IgG); the others are IgM, IgA, IgD, and IgE. Antibodies are responsible for "humoral immunity."

Antigen: A substance, usually a protein or complex carbohydrate, which, when introduced into the body of a human or other animal, stimulates the production of an antibody that reacts specifically with it.

Antigen probe: A sequence of DNA that is used to detect the presence of a particular nucleotide base sequence.

Antimalarial: A drug, such as chloroquine, that prevents or suppresses malaria infection.

Antiserum: Blood serum containing antibodies to a specific antigen.

Arboviral infections: A group of infections caused by arboviruses. Important human arboviral infections include yellow fever, dengue fever, oropouche fever, chickungunya, Japanese encephalitis, other viral encephalitides, and hemorrhagic fevers.

Arbovirus: One of a large number of viruses transmitted by or thought to be transmitted by arthropods (mosquitoes, ticks, etc.). The arboviruses do not comprise a natural taxonomic category of related organisms, but are grouped together because of their mode of transmission. Among them, however, are several groups of viruses that are closely related.

Arenavirus: One of a group of viruses traditionally grouped with the arboviruses, probably incorrectly. Arenaviruses probably are not transmitted by arthropods, though their life cycles still are not fully known.

Arthropod: An invertebrate animal belonging to the phylum Arthropoda, which includes insects, ticks, spiders, and crustaceans.

Attenuated vaccine: A vaccine made of whole pathogenic organisms that are treated with chemicals, radioactivity, or other means to render them incapable of producing infection.

Autoantibody: An antibody that is formed by an individual against the individual's own tissues.

Autoimmunity: An immune state in which antibodies are produced by an individual against his or her own tissue (autoantibodies).

Avirulent; nonvirulent: Capable of causing only a mild or inapparent infection.

Bacillus (pl., bacilli): Any of various rod-shaped, aerobic bacteria belonging to the genus *Bacillus*.

Bacillus Calmette-Guerin (BCG) vaccine: A vaccine prepared from attenuated *Mycobacterium bovis*, an agent that infects cattle, and used to immunize humans against infection with *M. tuberculosis*, the related bacterium that causes human tuberculosis.

Bacteremia: A pathologic state characterized by the presence of bacteria in the blood.

Bactericide: An agent capable of killing bacteria.

Bacteriology: The scientific study of bacteria.

Bacteriophage: One of a group of viruses that infects and replicates in certain bacteria. Also called "phage."

Bacteriostatic: Capable of slowing or halting the growth of bacteria without killing them.

Bacterium (pl., bacteria): Any of a group of one-celled micro-organisms having round, rodlike, spiral, or filamentous bodies that are enclosed by a cell wall or membrane and lack fully differentiated nuclei. Bacteria may exist as free-living organisms in soil, water, organic matter, or as parasites in the bodies of plants and animals. Some, but not all, bacteria can cause disease.

Base pairs (of nucleic acids): Nucleotide bases that pair across the double helix of the DNA or RNA molecule in a very specific way: adenine with thymine (or uracil in RNA), cytosine with guanine.

Biological control: The control of insects or other organisms necessary for the development or transmission of disease, through measures such as the introduction of natural predators or pathogens of the target organism, the use of naturally produced chemicals, and the introduction of sterile insects to the breeding population. (Compare *environmental control*.)

Bionomics: The study of the relationship of organisms to the environment.

Biotechnology: Techniques that use living organisms or substances from such organisms to make or modify a product. In this report, biotechnology refers to recombinant DNA techniques and other sophisticated tools relying on the ability to harness and manipulate genetic material.

B-lymphocytes: See *lymphocytes*.

Carrier: A person or animal who, without apparent symptoms, harbors a pathogen and may serve as a source of infection to others.

Case fatality rate: The proportion of individuals with a specific diagnosis who die from the disease during a specified period of time.

Cell culture: Growth in the laboratory of cells isolated from multicellular organisms. Each culture is usually of one type.

Cell-mediated immunity: Immunity resulting from increase of activity by living cells in the blood and other tissues (e.g., T-lymphocytes, natural killer cells) that directly and nonspecifically destroy foreign material. (Compare *humoral immunity*.)

Chagas' disease: Infection by *Trypanosoma cruzi*, transmitted by reduviid bugs. The disease was discovered and described by Carlos Chagas of Brazil. It is characterized by an acute course in children with fever, encephalitis, and inflammation of the heart muscle (often life-threatening or fatal), and a chronic course in adults leading to heart disease and heart failure. Also called "American trypanosomiasis." (See also *trypanosomiasis*.)

Chemoprophylaxis: The prevention of disease by the use of drugs or chemicals.

Chemotherapy: The use of specific chemical agents to arrest the progress of, or eradicate, disease in the body.

Cholera: A severe diarrheal disease caused by the bacterium *Vibrio cholerae*.

Chromosomes: The rodlike structures of a cell's nucleus that store and transmit genetic information; the physical structures that contain genes.

Chronic: Lingering, lasting, as opposed to acute.

Clone: A group of genetically identical cells or organisms produced asexually from a common ancestor.

Cold chain: The means whereby vaccines can be transferred from the manufacturer to the physician in the field at a sufficiently low temperature to ensure the effectiveness of the vaccines. Some of the important childhood vaccines require continuous refrigeration.

Complement: A protein complex in plasma that causes the lysis of bacteria and other pathogens that react with antibody.

Complementary DNA (cDNA): DNA that is complementary to messenger RNA; used for cloning or as a probe in DNA hybridization studies.

Congenital: Existing at birth.

Cross-reactivity: The property of an organism able to provoke an immunologic reaction against a different organism. The tuberculosis vaccine BCG, for example, is an attenuated strain of *Mycobacterium bovis* (a bovine tuberculosis) that provokes the immune reaction against *M. tuberculosis*, the cause of human tuberculosis.

DDT (dichloro-diphenyl-trichloro-ethane): An insecticide used to control mosquitoes and other vectors of tropical diseases.

Dengue fever: An acute febrile disease caused by an arbovirus, transmitted by mosquitoes of the genus *Aedes*, and characterized by fever, severe pains in the head, eyes, muscles, and joints, and a skin eruption.

Diagnostic technology: A technology used to determine the presence or absence of disease, and/or to characterize the extent of the disease.

Diarrheal diseases: Diseases characterized by the passage of loose watery stools, usually at more frequent than normal intervals. The dehydration that accompanies diarrhea is the cause of great morbidity and mortality, particularly among infants and children.

DNA (deoxyribonucleic acid): A nucleic acid, containing the sugar ribose, that is found in cell nuclei and is the carrier of genetic information. (Compare *RNA*.)

DNA-DNA hybridization: See *nucleic acid hybridization*.

DNA probe: A sequence of DNA that is used to detect the presence of a particular nucleotide sequence in a sample of DNA.

DNA sequence: The order of nucleotide bases in the DNA helix.

Dysentery: Inflammation of the intestine characterized by pain, intense diarrhea, and the passage of mucus and blood.

Electrolytes: Any compound that dissociates into charged ions in solution and can conduct a current of electricity. A balance of these in body fluids is necessary for the body to function normally.

Elephantiasis: The enormous swelling of a limb, usually a leg, as a result of lymphatic obstruction by filarial worms, followed by thickening of the skin and subcutaneous tissues.

Emesis: Vomiting.

Encephalitis: Inflammation of the tissues of the brain.

Endemic: Constantly present or persistent within a given geographic area, a term used to refer to a human disease or an infectious agent. (Compare *enzootic, epidemic*.)

Enteric: Pertaining to the intestine.

Enterotoxigenic: Capable of producing a toxin within the intestine. Some strains of *E. coli* cause disease by producing such a toxin.

Entomology: The scientific study of insects.

Environmental control: The control of disease through measures involving the alteration of the physical environment to eliminate the conditions necessary for the survival of the insects or other organisms that transmit or harbor the agents of disease.

Enzootic: The constant presence or persistence of an animal disease or infectious agent within a given geographic area; the animal counterpart of endemic. (Compare *endemic, epizootic*.)

Enzyme: Any of a group of catalytic proteins that are produced by living cells and that mediate and promote the chemical processes of life without themselves being altered or destroyed.

Epidemic: A sudden increase in the incidence rate of a human illness, affecting large numbers of people and spread over a wide area. (Compare *endemic, epizootic*.)

Epidemiology: The scientific study of the distribution and occurrence of diseases and health conditions, and their determinants.

Epitope: A structural part of an antigen that is responsible for an antibody response against that antigen. Also called "antigenic determinant."

Epizootic: Affecting many animals in one region simultaneously; the animal counterpart of epidemic. (Compare *enzootic, epidemic*.)

Erythrocytes: Red blood cells.

***Escherichia coli*:** A species of rod-shaped bacteria that inhabit the normal intestinal tract of vertebrates. Some strains cause intestinal disease and diarrhea in humans through at least three mechanisms: enterotoxigenic *E. coli* produces toxins that cause excessive fluid production in the intestine; enteroinvasive *E. coli* invades the cells of the intestinal wall; enteropathogenic *E. coli* produces a toxin that causes disease in infants. Many nonpathogenic strains of *E. coli* are used as hosts in recombinant DNA technologies.

Etiologic: Causative.

False negative: A negative test result in an individual with the disease in question, i.e., the patient is incorrectly diagnosed as not having a particular disease.

False positive: A positive test result in an individual who does not have the disease in question, i.e., the individual is incorrectly diagnosed as having a particular disease.

Fertility rate: The annual number of live births per 1,000 women of child bearing age (15 to 49 years) in a defined population.

Filaria: Parasitic nematode worms, named for their threadlike appearance.

Filariasis: A disease in humans due to infection with filarial worms, such as *Wuchereria bancrofti*, *Brugia malayi* (transmitted by mosquitoes), and *Onchocerca volvulus* (transmitted by blackflies). Adults of *W. bancrofti* and *B. malayi* live in the human lymphatic system and connective tissues, where they may cause obstruction. The immature worms (microfilariae) migrate to the host's bloodstream. Completion of the parasite's life cycle is dependent on passage through a mosquito.

Fluke: The common name for a large number of species of parasitic flatworms that form the class Trematoda.

Gamete: A mature germ cell with one set of chromosomes, capable of fusing with another gamete and forming a new, genetically distinct, individual.

Gametocyte: A life cycle stage of *Plasmodium*, the malarial agent; this stage infects mosquitoes after the mosquito bites an infected human (or other mammal), and gives rise to the gamete, or, sexual stage, of the parasite.

Gene: The basic unit of heredity; an ordered sequence of nucleotides comprising a segment of DNA. A gene contains the sequence of DNA that encodes one polypeptide chain (via RNA).

Genome: The genetic endowment of an organism or individual.

Genus (pl., genera): A taxonomic category that includes groups of closely related species of plants or animals.

Glycoprotein: A protein with attached sugar groups.

Helminth: Any parasitic worm.

Hemorrhagic fever: A severe complication of some viral diseases that involves internal or external bleeding. Several arboviruses sometimes cause epidemic outbreaks of hemorrhagic fever.

Host: 1. In the context of parasitology, a living organism that harbors a parasite. Definitive hosts harbor the adult or sexual stage of a parasite; intermediate hosts harbor the larval or asexual stages of a parasite. 2. In the context of recombinant DNA technology, the organism into which a scientist inserts foreign DNA.

Humoral immunity: Immunity associated with antibodies that circulate in the blood. (Compare *cell-mediated immunity*.)

Hybridoma: Product of fusion between a myeloma cell (which divides continuously in culture and is "immortal") and a lymphocyte (antibody-producing cell); the resulting cell grows in culture and produces the specific antibody produced by the parent lymphocyte (a monoclonal antibody).

Hypernatremia: An abnormally high concentration of sodium ions in the blood.

Hypnozoite: Forms of some species of *Plasmodium*, the cause of malaria, that remain dormant in liver cells, sometimes for many years, retaining their ability to activate an infection and cause acute malaria.

Hyponatremia: An abnormally low concentration of sodium ions in blood. Hyponatremia often accompanies severe diarrhea.

Immune: Protected against disease by innate or acquired immunity.

Immune response: The reaction of an organism to invasion by a foreign substance. Immune responses are often complex, and may involve the production of antibodies from special cells (B-lymphocytes), as well as a varying set of physical and chemical responses from other cells of the immune system.

Immune serum: Blood serum that contains antibodies and can be used to confer passive immunity to a variety of diseases.

Immune system: A specialized group of body cells and cell products that respond to foreign organisms and substances in the body. The cell products are largely immunoglobulins (antibodies). Some lymphocytes and various other cells of the immune system directly attack foreign organisms.

Immunity: A living organism's condition of being able or capacity to resist a particular disease. Innate immunity is natural or inherited. Acquired immunity may be active or passive. Active immunity results from previous exposure to the disease-causing agent or vaccination; passive immunity is the result of transfer of preformed antibodies in immune serum or from mother to fetus. (See also *cell-mediated immunity, humoral immunity*.)

Immunization: The deliberate introduction of an antigenic substance (vaccine) or antibodies into an individual, with the aim of producing active or passive immunity to a disease. (Compare *vaccination*.)

Immunization technology: Interventions designed to render individuals resistant to disease if they are exposed to a disease-causing agent. Vaccines are the most important tools of immunization.

Immunoassay: The use of antibodies to identify and quantify substances. The binding of antibodies to antigen, the substance being measured, is often accompanied by tracers such as radioisotopes.

Immunodiagnosis: A process whereby specified immunologic characteristics of cells, serum, or biologic specimens are determined for the purpose of diagnosing disease.

Immunogenic: Able to cause an immune response.

Immunoglobulin: Any of a set of serum glycoprotein molecules that have the ability to bind other molecules with a high degree of specificity. Immunoglobulins include all the antibodies.

Immunology: The scientific study of immunity, induced sensitivity, and allergy.

Immunosuppression: Suppression of the immunologic response.

Incidence: The frequency of new occurrences of disease within a defined time interval in a defined population. Incidence rate is the number of new cases of specified disease divided by the number of people in a population over a specified period of time, usually 1 year.

Incubation: The time between infection by a disease-causing organism and the appearance of disease.

Infant mortality rate: Number of deaths among children less than 1 year old as a fraction of the total number of live births in a year.

Infection: The entry and proliferation of any pathogenic organism in another organism.

Innate immunity: Immunity that is genetically determined at birth.

Inoculate: To introduce immunologically active material (e.g., an antibody or antigen) into, especially in order to treat or prevent a disease.

Insecticide: A substance capable of killing insects.

Insect vector: An insect that can transmit a disease-producing organism from one human or animal to another.

Integrated pest management (IPM): The use of a combination of biological, chemical, environmental measures to control vectors that transmit tropical diseases to humans or other animals.

Intermediate host: See *host*.

In vitro: Literally, in glass; pertaining to a biological process or reaction taking place in an artificial environment, usually a laboratory. Sometimes the term is used to include the growth of cells from multicellular organisms under cell culture conditions.

In vivo: Literally, in the living; pertaining to a biological process or reaction taking place in a living cell or organism.

Kinetoplastid: Characteristic structure at the base of the flagellum in certain protozoa (e.g., *Leishmania* spp. and *Trypanosoma* spp.).

Larvicide: A substance capable of killing insect larvae.

Leishmania: A genus of flagellated parasitic protozoans that cause leishmaniasis.

Leishmaniasis: Any of several infections caused by *Leishmania* spp., transmitted by sandflies. Cutaneous leishmaniasis is a skin ulcer caused by *L. mexicana* (New World) or *L. tropica* (Old World). Mucocutaneous leishmaniasis is an ulceration of the nose and throat caused by *L. braziliensis*, occurring in tropical America. Visceral leishmaniasis, also called "kala-azar," is a generalized and internal disease caused by *L. donovani* (New and Old World).

Lepromatous leprosy: See *leprosy*.

Leprosy: A chronic, infectious, granulomatous disease of humans caused by the bacillus *Mycobacterium leprae*. The disease occurs almost exclusively in tropical and subtropical regions, and ranges in severity from localized, spontaneously remitting lesions (tuberculoid leprosy) to malignant lesions with progressive anesthesia, paralysis, ulceration, nutritive disturbances, gangrene, and mutilation (lepromatous leprosy). Also called "Hansen's disease."

Lesion: A wound, injury, or one of the individual points or patches of a multifocal disease.

Lower respiratory tract infection (LRTI): See *acute respiratory infections*.

Lymphocytes: Specialized white blood cells involved in the body's immune response. B-lymphocytes originate in the bone marrow and when stimulated by antigen produce circulating antibodies (humoral immunity). T-lymphocytes originate in the thymus and engage in a type of defence that does not depend directly on antibody attack (cell-mediated immunity).

Lyophilized: Freeze-dried.

Macrophage: A type of large, ameba-like cell, found in the blood and lymph, which consumes foreign particles, including bacteria and parasites.

Malaria: Any of a group of human febrile diseases caused by infection of red blood cells by protozoan parasites of the genus *Plasmodium*, transmitted by mosquitoes of the genus *Anopheles*. Four species of *Plasmodium* cause malaria in humans: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. *P. vivax* and *P. ovale* have a persistent stage in the liver that causes relapses. Many other species of *Plasmodium* infect monkeys, rodents, birds, and reptiles.

Merozoite: A life cycle stage of the malarial agent *Plasmodium*; this stage develops in the vertebrate host's liver, then enters the circulatory system and infects red blood cells.

Messenger RNA (mRNA): RNA that serves as the template for protein synthesis; it carries the transcribed genetic code from DNA and directs protein synthesis.

Microfilariae: Slender, motile prelarval forms of filarial nematodes, the parasites that cause filariasis.

Micro-organism: A minute, microscopic, or submicroscopic living organism. Examples are bacteria, mycoplasma, viruses, and protozoa.

Molecular biology: The study of biology at the level of individual molecules, such as proteins and DNA.

Molluscicide: Any chemical agent used to kill mollusks; in the context of tropical diseases, snails necessary in the life cycles of schistosomes are the most important targets.

Monoclonal antibodies (MAbs): Homogeneous antibodies derived from clones of a single cell. MAbs recognize only one chemical structure. They are useful in a variety of industrial and medical capacities since they have remarkable specificity.

Monocytes: Phagocytic, large white blood cells, containing one nucleus.

Morbidity: The condition of being diseased.

Morphology: The study of the configuration or structure of organisms.

Mortality rate: The death rate, often made explicit for a particular characteristic, e.g., age, sex, or specific cause of death. A mortality rate contains three essential elements: 1) the number of people in a population group exposed to the risk of death; 2) a time factor; 3) the number of deaths occurring in the exposed population during a certain time period.

Mycobacterial diseases: A group of human and animal diseases caused by species of the bacterial genus *Mycobacterium*. Important human mycobacterial diseases are tuberculosis and leprosy.

Mycology: The scientific study of fungi.

Mycoplasma: Micro-organisms similar to bacteria, but lacking a rigid cell wall.

Nagana: A disease of cattle and other livestock in Africa, caused by *Trypanosoma brucei*, the cause of African sleeping sickness in humans.

Necrosis: Death of tissue.

Nematodes: Elongated, cylindrical worms, also called roundworms, many of which are parasites. Hookworm and the worms that cause trichinosis are nematodes. The filarial worms also belong to this group.

Nucleic acid: Macromolecules composed of sequences of nucleotides. There are two kinds of nucleic acids: DNA, which contains the sugar deoxyribose, and RNA, which contains the sugar ribose.

Nucleic acid hybridization: Matching of either DNA or RNA (depending on the organism) from an unknown organism with DNA or RNA from a known organism. This method is used in tropical disease research for identifying species and strains of pathogens.

Nucleotide: A structural unit of nucleic acid, consisting of a base, a sugar, and a phosphate molecule.

Oligonucleotides: Short segments of DNA or RNA, made up of a few (2 to 10) nucleotides.

Onchocerciasis: An infection of humans with the filarial worm *Onchocerca volvulus*, transmitted by the bite of blood-sucking blackflies. The disease is generally characterized by skin nodules that can become fibrous and calcified. Also called "African river blindness," for the blindness that occurs when the worms invade the eye.

Oral rehydration therapy (ORT): The treatment or prevention of dehydration due to diarrhea by a specific water solution of electrolytes and glucose (salts and sugar) taken by mouth.

Oropouche fever: An arboviral disease transmitted by biting midges (*Culicoides* spp.). Symptoms include anorexia, rash, and joint and muscle pain.

Orphan Drug Act: Public Law 97-414, which charges the U.S. Government with identifying and promoting orphan products, defined as drugs and devices for rare diseases.

Pandemic: Worldwide epidemic.

Parasite: An organism living in or on another living organism, obtaining from the host organism part or all of its organic nutriment.

Parasitemia: The presence of parasites in the blood.

Parasitic disease: A disease caused by a parasite. Examples are malaria, schistosomiasis, trypanosomiasis, leishmaniasis, and filariasis.

Parasitology: The scientific study of the relationship between parasites and their hosts.

Passive immunity: Immunity that is the result of the transfer of preformed antibodies in immune serum or from mother to fetus. (Compare active *immunity*.)

Pathogen: A specific causative agent (e.g., a virus or bacterium) of a disease.

Pathogenesis: The mode of origin and development of a disease process.

Pathogenicity: The condition of causing disease.

Pathology: The scientific study of the cause of disease, and the associated structural and functional changes that result from disease.

Pertussis: An infectious inflammatory respiratory disease of children caused by the bacterium *Bordetella pertussis*. The disease is characterized by explosive attacks of coughing ending in an inspiratory whoop. Also known as "whooping cough."

Phage: See *bacteriophage*.

Phagocytosis: Consumption of foreign particles (e.g., bacteria) by cells that use ameboid movement to surround the particle and then digest it.

Phenotype: The observable characteristics of a strain or species.

Phlebotomine sandflies: Insect vectors of *Leishmania* spp., the agents of leishmaniasis.

Plasmid: An extrachromosomal, self-replicating, circular segment of DNA. Plasmids can be used as "vectors" for cloning foreign DNA in bacterial "host" cells.

Plasmodium: The genus of protozoans that cause malaria in humans and other animals.

Pneumonia: An acute or chronic inflammation of the lungs, which can be caused by a variety of microorganisms.

Polypeptide: A sequence of amino acids (at least three) joined in a chain.

Prevalence rate: The number of *existing* cases of a disease in a defined population at a particular time, or over a specified time period.

Probe: See *DNA probe*.

Prophylaxis: The prevention of disease.

Protozoa: A phylum of one-celled animals, a few of which cause disease in humans. Examples are the causes of malaria, leishmaniasis, and trypanosomiasis.

Reagent: A substance that takes part in a chemical reaction.

Recombinant DNA: The hybrid DNA produced by joining pieces of DNA from different sources together in vitro.

Recombinant DNA techniques: Techniques that allow specific segments of DNA to be isolated and inserted into a bacterium or other host (e.g., yeast, bacteria) in a form that will allow the DNA segment to be replicated and expressed as the cellular host multiplies.

Recrudescence: The reappearance of a morbid process or its symptoms after a period of improvement.

Reduviid bug: A blood-sucking bug in the Reduviid family that is the vector of *Trypanosoma cruzi*, the agent of Chagas' disease.

Reservoir: Any person, animal, arthropod, plant, soil, or substance (or combination of these) in which an infectious agent lives in such manner that it can be transmitted to a susceptible host.

Respiratory syncytial virus (RSV): The most important cause of lower respiratory disease (pneumonia and bronchiolitis) in children under 2 years of age.

Restriction enzymes: Bacterial enzymes that cut DNA at specific nucleotide sequences.

Rhinovirus: One of many virus families that cause upper respiratory disease. Rhinovirus is a cause of the "common cold."

Rickettsia: A group of rod-shaped micro-organisms which may be transmitted by arthropods and are responsible for some human diseases such as Rocky Mountain spotted fever and epidemic typhus.

River blindness: See *onchocerciasis*.

RNA (ribonucleic acid): A nucleic acid, containing the sugar ribose, that is found in cytoplasm and some cell nuclei and is associated with the control of cellular chemical activities. In its three forms—messenger RNA, transfer RNA, and ribosomal RNA—RNA assists in translating the genetic message of DNA into the finished protein. (Compare *DNA*.)

RNA-RNA hybridization: See *nucleic acid hybridization*.

Rotavirus: Any of a group of viruses (round in shape) which are the major cause of diarrheal disease in infants and children.

Salmonella: A genus of bacteria that can cause diarrheal disease.

Schistosoma: The genus of blood flukes that cause schistosomiasis.

Schistosomiasis: A chronic, debilitating infection by worms of the genus *Schistosoma* ("blood flukes"). The three most important species in humans are: *S. mansoni*, *S. haematobium*, and *S. japonicum*.

Sensitivity: The ability of a diagnostic test accurately to diagnose a disease or condition when the disease or condition is present. High sensitivity means few false negatives. (Compare *specificity*.)

Serotype: A group of closely related micro-organisms that are distinguished by their possession of a common set of antigenic characteristics. The term also refers to the antigen set characteristic of such a group.

Serum: The clear liquid which separates in the clotting of blood and which contains the antibodies that were present in the whole blood.

Shigella: A genus of bacteria, some of which can cause diarrheal disease.

Species: A taxonomic category that includes closely related, morphologically similar individuals that actually or potentially interbreed; the principal subdivision of a genus.

Species complex: A group of two or more closely related species that can only be differentiated by cytogenetic analysis or cross-breeding experiments.

Specificity: The ability of a diagnostic test correctly to determine that an individual does not have a specific disease or condition. High specificity means few false positives. (Compare *sensitivity*.)

Sporozoite: A life cycle stage of the malarial agent *Plasmodium*; this is the stage injected by the mosquito vector into the vertebrate host's bloodstream.

Strain: A group of organisms of the same species having a distinctive quality or characteristic (biochemical, pathogenic, or other) that can be differentiated, but are not different enough to constitute a separate species.

Subunit vaccine: A vaccine that contains only portions of an antigenic molecule of a pathogen. Subunit vaccines can be prepared by using recombinant DNA technology to produce all or part of the antigenic molecule or by artificial (chemical) synthesis of short peptides.

Surveillance: Constant observation of an area to determine the level of disease activity.

TDR: The acronym for the Special Program for Research and Training in Tropical Diseases, sponsored jointly by the U.N. Development Program, the World Bank, and the World Health Organization.

TDR diseases: The six diseases singled out for attention by the Special Program for Research and Training in Tropical Diseases (TDR): malaria, schistosomiasis, trypanosomiasis, filariasis, leishmaniasis, and leprosy.

Therapeutic technology: A technology that cures or relieves the symptoms of a disease or other medical condition.

Titer: The lowest concentration (highest dilution) of an active substance (e.g., antibody in serum) that causes a discernible reaction with another substance.

T-lymphocytes: See *lymphocytes*.

Toxin: A substance, produced in some cases by microorganisms, which is toxic to other living organisms.

Transcription: The synthesis of messenger RNA on a DNA template; the resulting RNA sequence is complementary to the DNA sequence. This is the first step in gene expression. (Compare *translation*.)

Translation: The process in which the genetic code contained in the nucleotide base sequence of messenger RNA directs the synthesis of a specific order of amino acids to produce a protein. (Compare *transcription*.)

Transmission: The passage of a pathogen from one host to another host, or from vector to host.

Trematode: Any of a group of parasitic flatworms, including the flukes, of the phylum Platyhelminthes. Schistosomes are important human trematode parasites.

Trypanosoma: A genus of slender, polymorphic, parasitic protozoans that cause trypanosomiasis.

Trypanosomiasis: Any of several diseases caused by infection with species of the genus *Trypanosoma*. The important human diseases are African sleeping sickness (also called African trypanosomiasis) and Chagas' disease (also called American trypanosomiasis). African sleeping sickness is caused by *T. brucei rhodesiense* in east Africa or *T. b. gambiense* in west Africa, both transmitted by the tsetse fly. Chagas' disease is caused by *T. cruzi*, transmitted by blood-sucking reduviid bugs.

Tsetse flies: Any of several two-winged flies of the genus *Glossina* that occur in Africa south of the Sahara; medically important as vectors of African trypanosomiasis.

Tubercle bacillus: A bacillus causing tuberculosis; usually refers to *Mycobacterium tuberculosis*, the principal cause of human tuberculosis.

Tuberculoid leprosy: See *leprosy*.

Tuberculosis: A chronic infectious disease of humans and animals caused by any of several species of mycobacteria. It usually begins with lesions in the lung, but can spread to other parts of the body.

Upper respiratory tract infection (URTI): See *acute respiratory infections*.

Vaccination: The deliberate introduction of an antigenic substance (vaccine) into an individual, with the aim of producing active immunity to a disease. (Compare *immunization*.)

Vaccine: A preparation of living, attenuated, or killed bacteria or viruses, fractions thereof, or synthesized antigens identical or similar to those found in the disease-causing organisms, that is administered to produce or increase immunity to a particular disease.

Vaccinia virus: The organism that causes cowpox; its injection into humans results in immunity to the related smallpox virus.

Vector: A transmission agent: 1. In the context of medicine, a carrier of disease; usage commonly refers to arthropods (e.g., mosquitoes, sandflies, ticks) or rodents. 2. In the context of recombinant DNA technology, the DNA molecule used to introduce foreign DNA into host cells; vectors include plasmids, bacteriophages, and other forms of DNA.

Vector bionomics: The study of the habits (feeding, resting, and breeding) of vectors of disease and variations among different strains and in different locales.

Vector-borne disease: A disease transmitted by an insect or other vector. Such diseases include malaria, trypanosomiasis, and arboviral infections.

Vector control technology: A technology aimed at controlling the vectors that transmit disease or other organisms (e.g., snails) that are not true vectors, but serve as intermediate hosts of human or other animal disease organisms.

Vibrio cholerae: The bacterium that causes cholera.

Virology: The study of viruses.

Virulence: The degree and severity with which a pathogen is able to infect a population and cause disease.

Virus: Any of a large group of submicroscopic agents infecting plants, animals, and bacteria and characterized by a total dependence on living cells for reproduction and by a lack of independent metabolism. A fully formed virus consists of nucleic acid (DNA or RNA) surrounded by a protein or protein and lipid coat.

Water-borne disease: A disease transmitted through contaminated water. Most diarrheal diseases can be water borne.

Whooping cough: See *pertussis*.

Wild-type: The most frequently encountered phenotype in natural breeding populations.

Xenodiagnosis: A technique in which an intermediate host or vector is used to diagnose the presence of parasites in humans; e.g., reduviid bugs are permitted to feed on someone suspected of having Chagas' disease, and later, the bugs are examined for the presence of *T. cruzi* parasites.

Yellow fever: An acute febrile disease caused by an arbovirus that is transmitted by mosquitoes. Symptoms include a high fever, jaundice, black vomit, and anuria (absence of urine excretion). The virus that causes jungle/sylvan yellow fever is maintained in monkey reservoir hosts; urban yellow fever refers to transmission of the same virus to humans.

Zoonosis: A disease primarily of animals that is transmissible to humans under natural conditions.

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